PROCEEDINGS.

VOL. XXVII. MAY, 1930. NO. 8.

Pacific Coast Section.

University of California, Berkeley, April 12, 1930.

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Effect on Biological Activity of Substituting Sulphur for Oxygen in an Organic Arsenical Compound.

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Voegtlin and his associates[1] have called attention to the significance of sulphur metabolism in connection with arsenic toxicity. It seemed of interest to determine the effect on biological activity of substituting sulphur for oxygen in an organic arsenical compound where this substitution is possible. We approached this problem from our particular standpoint of the chemotherapy of amebiasis, determining toxicity on oral administration to cats and rabbits, and amebacidal activity *in vitro* on *Entameba histolytica,* employing technique described elsewhere,[2] except that we used a Musgrave-Clegg slant and Ringer's solution containing albumen and rice-starch with pH adjusted to lie between 7.4 and 7.6, in an effort to prevent absorption of the drug by the solid medium.

Through the courtesy of the Lilly Research Laboratories, we secured 4-carbaminophenyl-arsonic acid ($H_2O_3As.C_6H_4.NHCONH_2$) and 4-thiocarbamino-phenyl-arsonic acid ($H_2O_3As.C_6H_4.NHCS\text{-}NH_2$), which differ from each other in that the latter contains sul-

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* Lilly Research Fellow.

[1] Voegtlin, C., *et al.*, Pub. Health Reports, 1923, xxxviii, 1882.

[2] Anderson, H. H., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 267. Koch, D. A., *Univ. Calif. Pub. Zool.*, 1926, xxix, 241.

TABLE I.

Certain comparative physico-chemical and biologic data on carbamino-phenyl-arsonic acid and the corresponding thio compound, with data on sodium acetarsone for use as a standard in calculating therapeutic indices.

Drug	Actual arsenic content	Solubility in water pH=8.0	No. rabbits used	MLD in rabbits mgm./kg.	No. cats used	MLD in cats mgm./kg.	Amebacidal concentration, 48 hr.	Calculated therapeutic index
	%							
Sodium Acetarsone	25.3	1:12	15	150	10	125-150	1:600	1
Carbamino-phenyl-arsonic acid	26.9	1:100 (colloidal)	14	200	11	200-250	1:4000	8.8
Thiocarbamino-phenyl-arsonic acid	18.7	1:500 (colloidal)	8	more than 1000	15	700-800	1:600	5

phur instead of oxygen in the carbamide radicle. The actual arsenic content of the former agreed closely with theory, but there was only 18.7% arsenic in the thio compound instead of the theoretical 27.1%. It seems impossible to prepare this substance with an arsenic content much closer to the theoretical. The comparison of the two drugs was further complicated by the fact that the thio compound was much less soluble than the other even in alkaline solution.

We found, however, that thiocarbaminophenyl-arsonic acid is only about a fourth as toxic on oral administration to rabbits and cats as carbaminophenyl-arsonic acid, and that it is only about one-sixth as powerful an amebacidal agent *in vitro*. A summary of our data, with figures for sodium acetarsone as a standard for calculating therapeutic indices, is given in Table I. Our observations on the amebacidal power of sodium acetarsone agree with those of Sautet.[3]

It is important to determine whether or not a similar marked reduction in biological activity occurs when sulphur is substituted for oxygen in other pentavalent arsenicals, and also in trivalent arseno compounds where the arsenic is more electro-positive. In connection with Voegtlin's suggestion[4] correlating physico-chemical data on compounds like these with their biological activity, in particular that high water solubility leads to rapid excretion, resulting in low toxicity, it should be pointed out again that although carbamino-phenyl-arsonic acid is much more soluble than the corresponding thio compound, it is also much more toxic and it seems more generally biologically active.

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[3] Sautet, J., *Ann. Parasitol. Hum. Comp.*, 1927, v, 329.

[4] Voegtlin, C., *Physiol. Rev.*, 1925, v, 63.

4932

Effect of Codeine Phosphate by Mouth on Oxygen Consumption in Normal Humans.

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(Introduced by Chauncey D. Leake.)

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In studying the effects of drugs on basal metabolism, Boothby and Rowntree[1] reported two experiments with codeine in which they found 3% and 8% depression respectively of the basal metabolic rate one hour following the administration of a therapeutic dose of codeine (0.03 gm.). Investigating the effect of pre-anesthetic medicants on functional activity in normal humans, we found that morphine in oral doses ranging from 0.1 to 0.5 mgm. per kilo generally depresses within an hour the basal metabolic rate and tactile discrimination,[2] while pulse rate, interestingly, is increased but respiration is unaffected. In a similar study of the barbituric acid hypnotics we noted[3] a definite depression of oxygen consumption and tactile discrimination with the oral use of therapeutic doses of sodium di-ethyl barbiturate and of some of its common derivatives. Iso-amyl ethyl barbiturate and phenyl ethyl barbituric acid in ordinary doses, however, both increase the basal metabolic rate, but decrease the tactile discrimination. Codeine was next studied from the same aspect and the results of 10 experiments on 8 normal human subjects are reported here.

The determinations of oxygen consumption were made by the closed method with the Sanborn "graphic" apparatus and the Sanborn averages were used for estimating the basal metabolic rate. The subjects were trained healthy adults and the usual precautions were observed in making determinations. Codeine phosphate, in doses ranging from 0.2 to 1.0 mgm. per kilo, was dissolved in water and administered by mouth. The "normal" basal metabolic rate was estimated before administration of the drug and subsequent tests were carried out at 20, 40, and 75 minutes after the drug was given. Pulse rate and blood pressure determinations and tactile discrimination tests were made before and after the drug was given, while the respiratory rate was obtained from the "graphic" record.

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[1] Boothby, W. M., and Rowntree, L. G., *J. Pharmacol. Exp. Therap.*, 1923, xxii, 99.

[2] Anderson, H. H., Proc. Soc. Exp. Biol. and Med., 1929, xxvii, 102.

[3] Anderson, H. H., and Leake, C. D., in press.

TABLE I.
Maximum Functional Changes During One Hour's Observation Following Oral Administration of Codiene Phosphate in Normal Humans.

Subject	Sex	Age	Wt. kg.	Ht. cm.	Dose mgm./kg.	Resp. per min.	Pulse per min.	O_2 consump. cc. per min.	Tactile Discrim.
H.P.	F	28	62	165	0.2	+3	0	—13	—
M.C.	F	25	36.5	154	0.3	+1	—6	—13	—
E.C.	M	22	83	182	0.4	+3	0	—25	No change
M.P.	M	20	97	185	0.4	+2	—4	—15	Increase
E.C.	M	22	83	182	0.6	+2	—4	—20	No change
H.S.	M	23	66	172	0.6	+1	—8	—10	Decrease
M.F.	M	22	63	160	0.8	+2	0	—29	Increase
E.J.	M	22	66	175	0.8	+1	0	—10	Decrease
H.P.	F	28	62	165	1.0	+2	—4	—13	”
H.A.	M	26	70	170	1.0	+3	0	—30	”

Table I shows the results of this study. Although the effect on the basal metabolic rate was not marked there was uniformly a depression of 4% to 13% following the drug. The extent of depression seemed independent of the dosage in the range we used. In most cases the basal rate returned to "normal" within 75 minutes after the drug was given. There was, surprisingly, an increase of respiratory rate in every case. The pulse rate was decreased when affected. Changes in blood pressure and tactile discrimination were inconstant. A tendency to sleep was noted only in the highest doses used.

Codeine, even in twice the dosage of morphine, is not nearly as powerful a depressant agent in the normal human. It is often said, on the authority of Issekutz,[4] who worked on animals, that the effects of morphine derivatives, including codeine, are essentially the same on respiration as those of morphine. Again, Macht[5] stated that morphine and, to a lesser degree, codeine are sedative or depressant to the respiratory center. During the first hour after administration in normal humans, however, codeine definitely and consistently seems to stimulate the respiratory rate in normal humans, while we failed to note any such effect following morphine. Another interesting difference between the effects of morphine and codeine is on pulse rate after administration of therapeutic doses to normal humans. Morphine tends within an hour to increase the heart rate while codeine tends to slow it.

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[4] Issekutz, B., *Arch. f. d. ges. Physiol.*, 1911, cxlii, 255.

[5] Macht, D., *J. Pharmacol. Exp. Therap.*, 1915, vii, 339.

4933

Comparative Diuretic Actions of Bismuth, Digitalis and Theophylline; Changes in Blood and Urinary Metabolites in Edema.

A. B. STOCKTON. (Introduced by P. J. Hanzlik.)

From the Departments of Pharmacology and of Medicine, Stanford University School of Medicine, San Francisco, California.

The diuretic action of bismuth has now been demonstrated in a total of 21 patients with edema, thus confirming the results previously reported.[1] Attempts are being made to determine, if possible, the mechanism of the diuretic action from comparisons with the well-known diuretics, digitalis and theophylline, and chemical analyses of blood and urine. The results on 10 patients with edema are definite enough to warrant a report at this time.

The edemas represented were mostly anasarca of cardiac origin, nephrosis, and ascites due to hepatic cirrhosis. The patients were at complete rest in bed on constant fluid and salt intakes throughout. Urine was collected during 24-hour periods and samples of blood were removed daily, or as often as necessary, for analysis. Urea of the blood and urine was estimated by Folin's method[2]; chlorides of the blood by Austin and Van Slyke's method[3]; chloride of the urine by the Seelman-Volhard method,[4] and hemoglobin by Palmer's method.[5] After suitable control periods, at least 2 and sometimes all 3 of the following drugs were administered successively with intervening periods without medication; bismuth sodium tartrate (Searle), 0.03 to 0.06 gm. intramuscularly; powdered (standardized) digitalis in capsules, 0.4 to 2.5 gm. orally; and theophylline sodium acetate, 1.2 gm. orally, total doses. Generally, the bismuth was used after the other diuretics had been tried.

Briefly summarized, the results obtained in 8 patients showing definite diuretic action were as follows: The maximum increase in daily urine output after digitalis was from 100% to 190% of the control output; after theophylline, from 30% to 280%; and after bismuth from 10% to 360%. The duration of the diuresis after digitalis was about 4 days, after theophylline 1 day, and after bismuth 4 days (medians). The removal of edema fluid was estimated to be from 930 to 3300 cc. after digitalis, from 70 to 1750 cc. after theophylline, and from 200 to 3900 cc. after bismuth. Thus, the diuretic and antiedemic efficiencies of bismuth compared favorably with, or were superior to, those of the other drugs. Bismuth was more efficient when compared with digitalis and theophylline used individually, but less efficient when compared with the latter two drugs used together.

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[1] Hanzlik, P. J., Bloomfield, A. L., Stockton, A. B., and Wood, D. A., *J. Am. Med. Assn.*, 1929, xcii, 1413.

[2] Folin, O., *Z. f. Physiol. Chem.*, 1901, xxxii, 504.

[3] Austin, J. H., and Van Slyke, D. D., *J. Biol. Chem.*, 1920, xli, 345.

[4] Seelman, J. J., *J. Lab. Clin. Med.*, 1916, i, 444.

[5] Palmer, W. W., Proc. Soc. Exp. Biol. and Med., 1914, xii, 175.

Bismuth and theophylline (digitalis not being as extensively investigated) caused concurrent, definite, and sometimes marked, increases in blood- and urinary-chlorides and an increase in urine-urea with a simultaneous decrease in blood-urea, when increases in diuresis resulted. These correlations failed of demonstration in 2 moribund patients who did not respond to bismuth and theophylline. Accordingly, the results as a whole fit in with modern conceptions of the mechanism of action of purine and certain metallic diuretics, namely, that the diuretic action is mediated through the tissues as a whole. The well-sustained diuretic and antiedemic actions of bismuth are more consistent with a tissue than with a purely renal action.

Final conclusions are reserved, however, until other possibilities are explored, and the diuretic and antiedemic efficiencies of various diuretics are tested in different clinical edemas.

4934

A Receiver Unit for the Determination of Lactic Acid.

JOHN FIELD 2D. (Introduced by E. G. Martin.)

From the Laboratories of Physiology, Stanford University, California.

The analytical methods for determination of lactic acid have been greatly improved in the last few years, notably by Clausen[1]; Friedmann, Cotonio and Shaffer[2]; Davenport and Davenport[3]; and Friedemann and Kendall.[4]

The receiver unit here described was designed for use with the

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[1] Clausen, S. W., *J. Biol. Chem.*, 1922, lii, 263.

[2] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, lxxiii, 335.

[3] Davenport, H. A., and Davenport, H. K., *J. Biol. Chem.*, 1928, lxxvi, 651.

[4] Friedemann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, lxxxii, 23.

Friedemann, Cotonio and Shaffer[2] apparatus and procedure. When small amounts of lactic acid are to be determined, better checks have been obtained by this method than by the use of other types of receivers. A marked advantage lies in the fact that its use involves a minimum of dilution of the receiver contents, through simplification of washing. This is important, because the end point of the iodine titration is sharper the lower the volume of liquid titrated.

The receiver consists essentially of a 5/16" x 6" test tube, carrying a 2-hole rubber stopper. Through one of these holes there runs a glass tube, drawn into a capillary as soon as it passes the stopper, and extending to the bottom of the tube. The other hole carries a trap, which leads to the source of suction.

In an analysis each such receiver contains 10 ml. of 1.0% sodium bisulphite. Accordingly the incoming gases are drawn through a column of bisulphite at least 10 cm. high, in the form of a stream of small bubbles. The fineness of these bubbles insures a large ratio of surface area to volume, which favors absorption. If the suction be properly regulated, no liquid will be drawn up into the trap, yet a steady stream of bubbles can be maintained.

At the end of a run the tubes are emptied into 125 ml. pyrex Erlenmeyer flasks, the receiver is washed out twice with 2 ml. of wash water, and the titration is carried out in the usual way. As the addition compound has not been decomposed at this point, the oxidation of some of the bisulphite consequent upon this pouring is not a serious matter.

Seventeen runs on aliquots of zinc lactate containing 0.64 mgm. lactate ion resulted in an average recovery of 92.0% and a median of 93.9%. Nine of the runs were above 90%, these averaged 97.3%, and 14 were above 85%, averaging 94.7.

The trial runs clearly showed that it is more accurate to use the higher value of a pair of results than it is to average the two, since only one value of the 17 exceeded 100.0%.

4935

Distribution of Lactate Between Corpuscles and Plasma in the Dog.

JOHN FIELD 2D. (Introduced by E. G. Martin.)

From the Laboratory of Physiology, Stanford University, California.

In connection with an extensive series of studies on metabolism after anoxemia now in progress in this laboratory[1] it was found desirable to determine whether or no lactates were distributed evenly between the corpuscles and plasma of the dog.

It has long been known that a corpuscle plasma concentration gradient obtains for several substances. In 1867 Zuntz[2] showed that the serum of normal blood contains about twice as much carbon dioxide (bicarbonate) as the corpuscles. Gürber[3] found that chlorides and bicarbonates move in and out of the corpuscles as the carbon dioxide tension of the gases in contact with the blood changes, and Van Slyke and Cullen[4] demonstrated this shift with changes in carbon dioxide tension lying in the physiological range. The partition of fermentable sugar between corpuscles and plasma is unequal[5] and there is a well demonstrated corpuscle-plasma gradient of pH,[6] pNa and pK.[7]

In a series of 6 experiments on resting amytalized dogs, determinations were made of the lactate content of plasma and of whole blood by the method of Friedemann, Cotonio and Shaffer.[8] The following values were obtained.

Exp. No.	$\frac{\text{plasma lactate concentration}}{\text{whole blood lactate concentration}}$
I- (1)	1.42
H- Bl. 3	1.50
H- Bl. 4	1.68
H- Bl. 22	1.39
H- Bl. 23	1.49
H- Bl. 24	1.39

The arithmetic mean of these ratios is 1.48.

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[1] Martin, E. G., Field, John II, and Hall, V. E., *Am. J. Physiol.*, 1929, lxxxviii, 407.

[2] Zuntz, H., *Centralblatt. Med. Wiss.*, 1867, p. 529.

[3] Gürber, A., *Maly's Jahresbericht.*, 1895, xxv, 164.

[4] Van Slyke, D. D., and Cullen, W. D., *J. Biol. Chem.*, 1917, xxx, 289.

[5] Somogyi, M., *J. Biol. Chem.*, 1928, lxxviii, 117.

[6] Hampson, A. C., and Manzels, M., *J. Physiol.*, 1927, lxiv, Proceedings.

[7] Van Slyke, D. D., *Physiol. Rev.*, 1921, i, 141.

[8] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, lxxiii, 335.

In 4 experiments in this laboratory it was found that the corpuscles constitute 47.3% of the blood volume. This agrees fairly well with the mean value of 45.21% found by Powers, Bowie and Howard on a series of twenty-five dogs.[9]

Assuming that the corpuscles constitute 45.2% of the blood volume, calculation from the above data gives a value of 3.34 for the ratio

$$\frac{\text{plasma lactate concentration}}{\text{corpuscle lactate concentration}}$$

While the finding is chiefly of qualitative interest, owing to the statistical inadequacy of the data, it is clear that there is a marked inequality in the distribution of lactate between the corpuscles and plasma of the dog.

4936

Dilatation of the Heart by Amytal.

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From the Division of Physiology, University of California Medical School, Berkeley, California.

Amytal, prepared as for surgical anesthesia, was added to the venous reservoir of an open-circuit heart-lung-preparation. The preparation was made under pure ether anesthesia, and in each case the experiment was postponed for at least 45 minutes for the effects of the ether to wear off. Most of the blood was defibrinated and the remainder rendered incoagulable by the addition of 25 mgm. heparin. The venous pressure and artificial resistance pressure were maintained constant; heart volume changes were recorded by a float recorder connected to a Jerusalem and Starling cardiometer. The useful (non-coronary) output of the heart was measured several times before and after each dose by collection in a graduated cylinder for 10 second periods. At the conclusion of each experiment the quantity of blood in the reservoir, cannulae and apparatus was measured and the heart and lungs were weighed and measured by displacement. The resulting estimate of quantity of tissue is probably accurate to within 10%.

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[9] Powers, J. H., Bowie, M. A., and Howard, I. M., *Am. J. Physiol.*, 1930, xcii, 665.

TABLE I.

	Exp. No.	Dose	Diastolic vol. after dose	Useful output cc. per min.	Rate	Stroke volume
			Amytal			
Dog 1	1	18 mgm./kilo	+	360 cc. No change	No change	Inc.
	*2	27 " / "	++	360-300	" "	"
	*3	45 " / "	+++	300-275	" "	"
Dog 2	4	50 " / "	+	280-264	" "	"
	*5	50 " / "	++	280-188	" "	"
Dog 3	6	58 " / "	++++	264-66-0 Heartblock	" "	"
Dog 4	7	70 " / "	+++	547-360-180	" " (120-120)	Dim.
	8	70 " / "	Very slight increase	210-60	No change	"
Dog 5	9	20 " / "	+	No change	(No venous pulsation)	
	10	50 " / "	++++	Flow stopped	(Venous pulsation)	
		Washed carefully—adrenalin—almost complete recovery				
	11	75 " / "	++++	Flow stopped	(Venous pulsation)	
			Ether			
Dog 6	12	2 breaths	+++	250-210	No change	Inc.
	13	3 "	++++	Dim.	130-119	"
	14	3 "	++++	"	120-60	"
	15	Greater than 3 breaths	+++++	250-60	No change	"

* Before these experiments blood was drained from venous reservoir and discarded; fresh blood added to wash out tissue, and discarded; more fresh blood added for experiment.

The table gives a record of our experiments comparing the effects of amytal with those of ether, the former being expressed in mgm. amytal per kilo of tissue (heart, lung, blood), the latter in the number of breaths during which a wad of cotton soaked in ether was plugged into the intake pipe of the artificial respiration pump.

Fig. 1 shows a typical cardiometer tracing, recording the result of a dose of 27 mgm. amytal per kilo. With this slight dose, as with all other doses of amytal, save in one case where the heart was revived by adrenalin, no recovery was observable during at least half an hour although fresh blood was substituted twice. According to Eddy[1] such a dose has no anesthetic effect; on the contrary the sensitivity to pain is heightened.

Examination of the results in the table shows that after a very small dose of amytal (18 mgm. per kilo) an increase in diastolic volume takes place although the venous pressure be maintained prac-

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[1] Eddy, N. B., *J. Pharm. and Exp. Therap.*, 1928, xxxiii, 43.

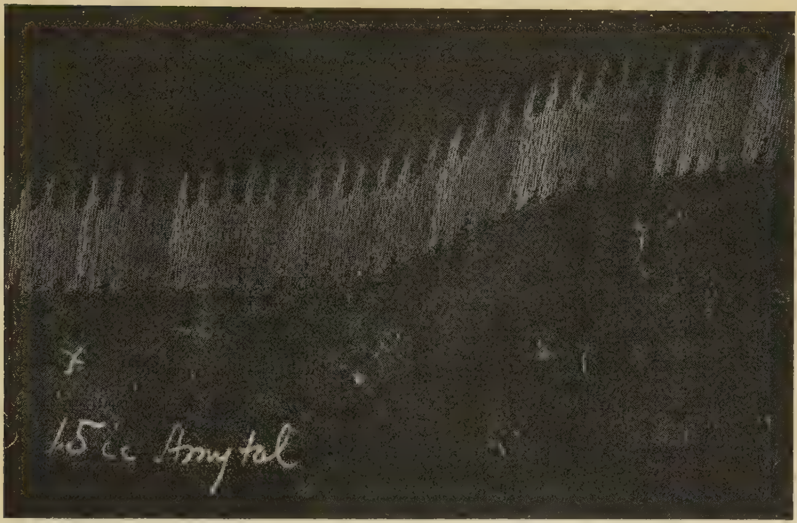

FIG. 1.

tically constant. Presumably dilatation is necessary to enable the heart to maintain its useful output. A greater dose causes a greater dilatation which, however, is accompanied by a diminution in useful output, since the dilatation is apparently not sufficient to compensate for the effect of the drug. This diminution in output may be observed with a dose as small as 27 mgm. per kilo, which is about half that commonly used for anesthetic purposes. It should, however, be remembered that in a whole animal under anesthesia a rise in venous pressure is possible and would provide further scope for compensation, so that there might be no diminution of heart output. In one experiment after a small dose, we raised the venous pressure, and found that compensation was perfect.

A dose of ether of 3 breaths produced the same effects in a more marked degree than the usual anesthetic dose of amytal, but with ether the effects rapidly passed off (*i. e.,* diastolic volume had returned to normal within 3 minutes). Ether in large doses sometimes produced a marked diminution in heart rate. With amytal we were able to observe a change in rate only in the one case where we used so large a dose as to produce heart block and death. Eddy[1] found a diminution of heart rate in intact cats with all doses of amytal from 25 mgm. per kilo up.

Accurate quantitative determinations of stroke volume have not yet been made, but in all cases save one, an increase of stroke volume was observed to accompany the diminished useful output. This suggests that one action of the drug may be a relaxation of the coronary system, though if an increase in coronary flow takes place, we have no indication whether this is in response to some change in the myocardium or is itself the primary effect of the drug. We have observed marked venous pulsation when the outflow is almost completely stopped, but since increasing the venous pressure will compensate for considerable diminution in flow without producing venous pulsation, we do not believe that valvular incompetence plays any part in the diminution in flow except with abnormally large doses, in which case the flow is almost *nil*.

Conclusion. In a heart-lung-preparation amytal increases diastolic volume and decreases useful outflow.

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Changes in Temperature of the Lower Extremities Following the Induction of Spinal Anesthesia.*

SELLING BRILL AND LESTER B. LAWRENCE. (Introduced by C. D. Leake.)

From the Department of Surgery and the Pharmacological Laboratory of the University of California Medical School, San Francisco.

We were interested to determine whether or not spinal anesthesia would cause vasodilatation and increased blood flow in the lower extremities with a resulting rise in temperature, and whether or not such effects of blocking the sympathetic nerves would be of aid in selecting patients for surgery of the sympathetic nervous system.[1]

Temperature readings were taken with the thermo-electric apparatus designed by Bazett and McGlone,[2] which contains loop and needle thermocouples allowing accurate surface and tissue temperature measurements to be made on human subjects. Surface readings were taken from the plantar surfaces of the big and little toes of both feet and from the palmar surfaces of the index, middle and ring finger of one hand. Tissue temperatures were noted by inserting the needle thermocouple from 1 to 2 cm. into the first and fourth metatarsal spaces of both feet. One or more readings were taken before induction of spinal anesthesia and frequent observations were made for 40 or more minutes afterward. Procaine hydrochloride

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* Aided in part by a grant from the Purington Research Fund.

[1] This problem arose from certain suggestions of Prof. H. C. Naffziger.

[2] Bazett, H. C., and McGlone, F., *J. Lab. and Clin. Med.*, 1927, xii, 9, 913.

in doses of 120 mgm. was used as the local anesthetic agent. At the same time careful record was kept of the room temperature, mouth temperature, blood pressure, and the extent of the anesthesia. Observations have been made on 6 male patients in whom there were no circulatory disturbances, but 3 of whom had 50 mgm. of ephedrine sulphate subcutaneously 30 minutes to 2 hours before coming to the operating room.

The surface temperature observations may be criticized because of difficulty of controlling extraneous conditions of temperature, hu-

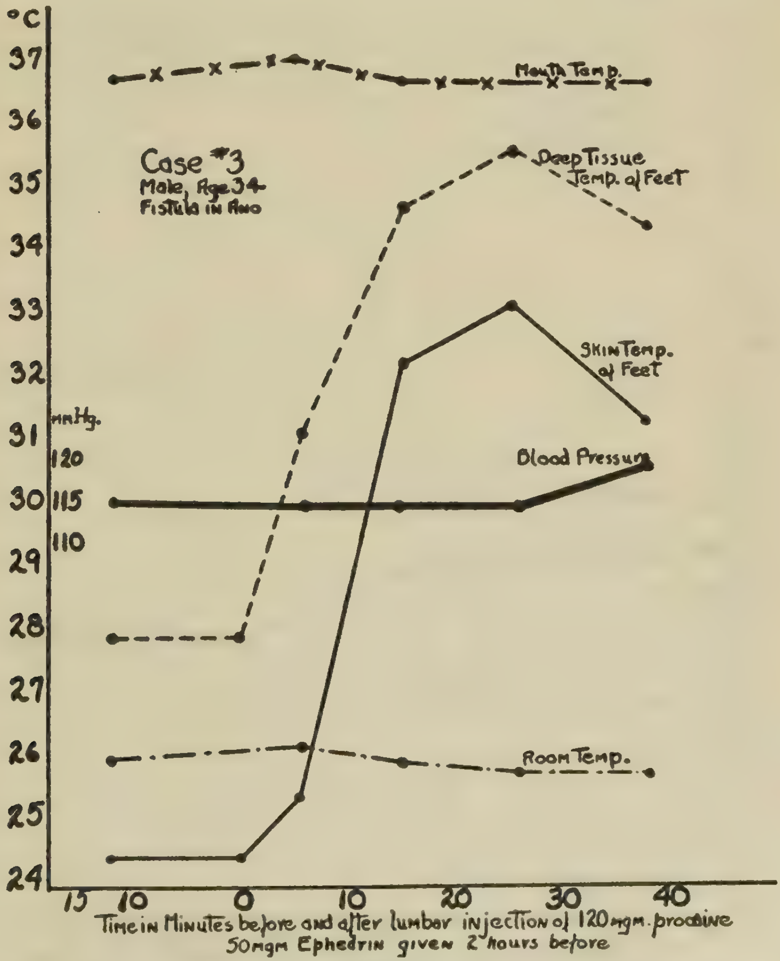

FIG. 1.

Graphic representation showing effects of the intraspinal administration of procaine hydrochloride on skin and tissue temperatures of feet in a patient with no circulatory disturbance.

midity, and air currents in the operating room. Changes in position and covering brought about by moving the patient for the administration of the anesthetic also affect the surface temperature. We believe that the deep tissue temperatures are little altered by these factors.

As may be seen by examination of some of our data, submitted graphically for conservation of space, both skin and deep tissue temperatures of the feet rise rapidly after induction of low lumbar spi-

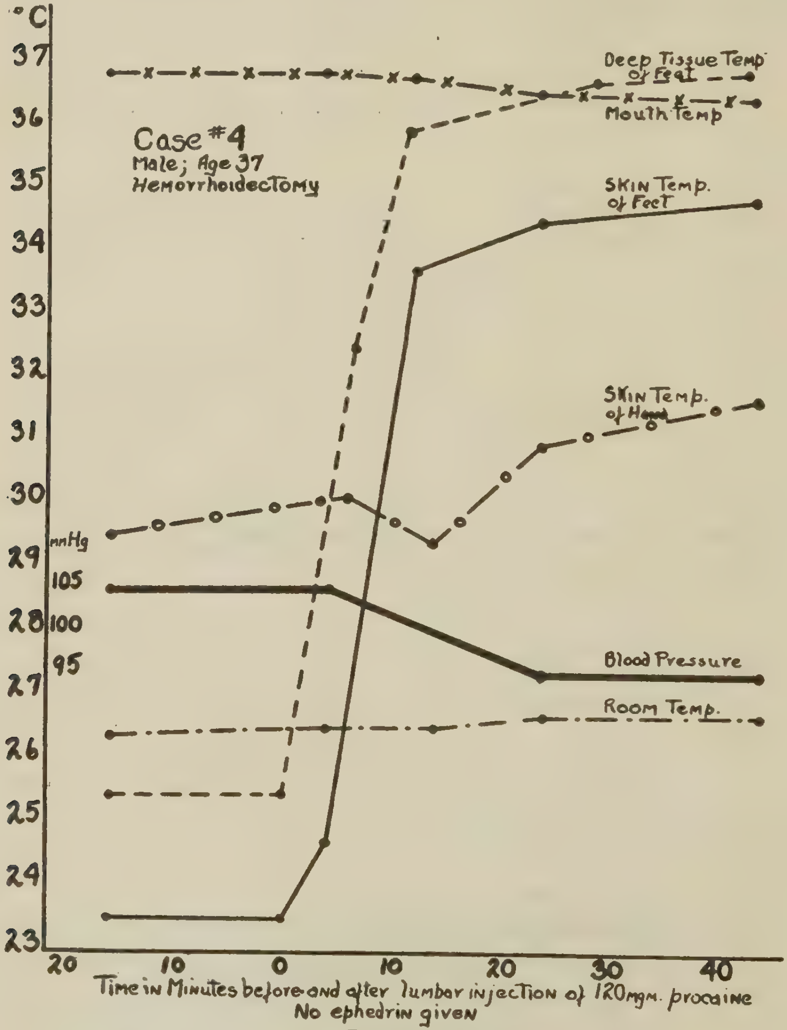

FIG. 2.

Graphic representation of effects of spinal anesthesia on skin and tissue temperatures of feet in comparison with temperature changes elsewhere in a patient with no circulatory disturbance.

nal anesthesia. In patients who are placed on their backs immediately following the administration of the local anesthetic, there is approximately equal anesthesia and an almost equal rise in temperature in both legs, although in one instance there was a difference of 2°C. We noted in 3 cases a drop in the hyperthermia of the extremities occurring with return of sensation.

Summary: Direct observations by accurate technique show that skin and deep tissue temperatures of the feet rise markedly following the induction of spinal anesthesia and begin to fall again as sensation in the feet returns. This may be interpreted as a result of vasodilatation in the feet following chemical blocking in the spinal canal of the significant nerves controlling vascular tone in the lower extremities.

Missouri Section.

Washington University School of Medicine, April 9, 1930.

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Studies on the Nature of Bacterial Hemolysin and Proteolysin.

L. R. JONES. (Introduced by M. S. Fleischer.)

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Many cultures of staphylococci and colon bacilli recently isolated from lesions are hemolytic when grown on blood containing media and usually such organisms are proteolytic when grown in nutrient gelatin. It has not been determined whether the hemolysin is identical with or produced parallel to the proteolytic substance. Both qualities are frequently transient and may exhibit a parallel decline. An attempt was made to study these substances with reference to individuality, interdependence or homologous nature.

Filtrates of broth cultures were tested for hemolytic activity as follows: measured quantities of filtrate were added to tubes containing 1 cc. each of a 0.5% suspension of washed red blood cells, incubated at 37°C., for 1 hour, placed in the refrigerator over night and the amount of lysis observed. Proteolytic activity was determined by streaking filtrate upon the surface of an agar-gelatin medium, incubating at 37°C., for 48 hours and testing for lytic action by flooding the plate with an acid bi-chloride of mercury solution according to the method of Frazier[1] and noting any change in the character of the gelatin as indicated by a clear zone at the site of "planting".

Filtrates of 4 to 8 day broth cultures of a proteolytic and hemolytic, Gram negative, motile organism, of intestinal origin and considered as belonging to the colon group were very active in lysing red blood cells and in digesting gelatin.

In filtrates heated to 55°C., for 5 minutes the hemolysin was destroyed, while the proteolysin was destroyed only after 30 minutes at that temperature.

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[1] Frazier, W. C., *J. Inf. Dis.*, 1926, xxxix, 302.

In absorption experiments it was found that by incubation of the active filtrate with red blood cells at low temperature it was possible to absorb and remove all of the hemolysin without marked decrease in proteolytic activity of the filtrate.

Experiments were also made with a proteolytic and hemolytic culture of *Staphylococcus albus,* isolated from a case of middle ear disease. Difficulty was encountered in preparing an enzymatic material containing both the hemolytic and proteolytic substances as filtrates of plain broth cultures were devoid of proteolysin and contained the hemolytic principle only irregularly. Broth cultures of various ages sterilized by addition of thymol were active in lysing red blood cells but were without effect upon gelatin. Living organisms as obtained from agar cultures, suspended in saline solution, manifested the hemolytic property. However, hemolysin production was probably coincident with bacterial activity during the hour of incubation at 37°C.

By sectioning cylinders of agar media below the surface growth of the organisms and extracting with salt solution the hemolytic substance was found to have diffused as far as 1.5 cm. below the surface culture. However, proteolysin could not be demonstrated in these sections.

The hemolysin in broth filtrates was most active in lysing red blood cells of the rabbit, less active toward those of the sheep and guinea pig and least active with those of man.

Dilution of the hemolytic filtrate with saline solution 1:10 did not interfere with activity. However, dilution with sterile broth 1:2 decreased lytic activity and inhibition was complete at a dilution of 1:10. Exclusion of air by covering the mixture of cells and filtrate with oil served to delay the appearance of lysis and quantitatively reduced lytic activity. Heating the filtrate to 50°C., for 40 minutes decreased lytic activity while at 56°C. for 10 minutes, hemolysin was entirely destroyed.

In later experiments, filtrates were made from 3 to 6 day cultures in proteose-peptone broth (pH 7.5) containing 0.5% of aminoids and sterilized by Seitz filtration. These filtrates contained hemolysin and proteolysin. It was possible to absorb and remove all of the hemolysin by incubating mixtures of filtrate and cells for 1 hour at 5°C. Cells sensitized in this manner and removed by centrifuging lysed upon re-suspending in salt solution. The supernatant fluid contained proteolysin but was devoid of hemolytic activity.

By forcing the dissociation of the original culture, 2 aberrant types were obtained upon subculture, one hemolytic and non-proteolytic, while the other was proteolytic and non-hemolytic.

The data indicate that hemolytic and proteolytic activities of filtrates prepared from cultures of both of these organisms are manifested by individual extracellular substances, and that hemolytic and proteolytic properties of viable staphylococci may be independently altered by means of bacterial dissociation.

4939

A Useful Modification in the Preparation of Therapeutic Sera.

J. BRONFENBRENNER.

From the Department of Bacteriology and Immunology, Washington University Medical School, Saint Louis, Missouri.

There are two recognized categories of individuals who are potentially subject to acute complications incident to serum therapy. One group is composed of those naturally hypersensitive to horse protein. The other is composed of individuals who are so constituted that they become hypersensitive to horse protein on the first exposure, and henceforth react like the individuals of the first group to the injection of the horse serum. (The latter category of individuals is likely to increase in the future due to toxin-antitoxin immunization.) While accidents of this sort can be avoided by the use of therapeutic sera derived from such animals to the protein of which the recipients show no hyper-susceptibility, in practice this procedure can not be carried out because therapeutic sera are prepared in horses almost exclusively. It is evident that if the protein of the therapeutic serum could be deprived of its species specificity the difficulty might be solved. The work of Obermeyer and Pick, of Landsteiner and of others has shown that by subjecting proteins to azotization, acetylation, halogenation, or by coupling with carbohydrates or lipoids, it is possible to destroy the species specificity of proteins while imparting to them new artificial specificities. In the past these experiments were made only with the view of eliciting the interdependence between the chemical structure and antigenic specificity. Accordingly chemical procedures selected were often so drastic that they would not be applicable to the problem at hand without some modification. By excluding such procedures which are accompanied by excessive oxidizing or reducing processes, or those in which the temperature or hydrogen-ion levels are not compatible with the preservation of specific properties residing in the serum proteins encouraging results have been secured. Therapeutic sera derived from horses treated under these conditions retained considerable portion of therapeutic value, and yet they did not cause anaphylaxis when injected into guinea pigs sensitized to native horse serum. Thus far only two mutually heterologous couplings have been secured but the experiments are being continued in the search for further and simpler procedures in order to secure sufficient variety of coupled sera so that 3, 4 and more reinjections of therapeutic sera may be made without the danger of shock. At the same time the couplings already secured are being tested for the toxicity when injected into animals in large amounts.

4940

Some Chemical and Physical Properties of the Crystalline Follicular Ovarian Hormone: Theelin.

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From the Laboratory of Biological Chemistry, St. Louis University School of Medicine.

The quantity of the earlier preparations of the crystalline hormone was insufficient for much chemical work. Three samples which were carefully assayed showed a potency exceeding 3000 rat units per milligram. The melting point of the crystals of preparations No. 119 and No. 143 was 243°C. (uncor.).

When larger quantities of the crystals became available an investigation of the chemical and physical properties of the compound was begun. The data given in Table I were obtained by micro methods which had been studied with known compounds.

The melting point was taken by the open beaker method. The corrected temperature is approximately 6° higher. The molecular weight determined by the Rast micro method gives values which agree with the values calculated from the Iodine number upon the assumption that there is one double bond in the molecule. The Iodine number was determined at 0°C. in the dark by a micro modification of the Hanus method. The hormone was acetylated with acetic anhydride in pyridine and the weight obtained indicated two hydroxyls. The molecular weight of the recrystallized derivative

TABLE I.

Preparation No.	Melt. Point (Uncor.)	Molecular Weight		Iodine No.	Hydroxyl		
		Rast	From I. No.		No.	Diacetyl Derivative	
						Mol. Wt.	Melt. Point
F153	243°	277,284,295	273	92.9	2	359	122°
F156	243.2°	268,269	264	96.1	2	353	122°
A160	243.5°	272,276	*	*	*	*	*
164	243.5°	267,268,270	262	96.7	*	*	*
Average		274.6	266				

* Not run on this sample.

confirmed this. The esterification of 2 alcoholic radicals would increase the molecular weight by 84 gm. which, added to the average value obtained by the Rast method ($274 + 84 = 358$), gives a value close to those experimentally determined.

The crystals were analyzed for C and H by Pregl's micro combustion method. Average, C 79.69%; H 8.49%; O 11.82%. These data give an empirical formula of $C_{18}H_{23}O_2$ with a molecular weight of 271, which corresponds with the data of the table.

4941

The Use of Plasma in the Kline Test for Syphilis.

K. L. BURDON AND L. BROMBERG. (Introduced by J. Bronfenbrenner.)

From the Department of Bacteriology and Immunology and the Department of Internal Medicine, Washington University Medical School, St. Louis, Mo.

Among the several successful precipitation methods for the diagnosis of syphilis recently developed the Kline microscopic slide test has been found to be one of the most satisfactory. Though more sensitive than other tests it is nevertheless highly specific.

Considerable time is usually expended in the collection and separation of the patient's serum, even though the performance of the test itself may be accomplished within 5 minutes. At the suggestion of Dr. Bronfenbrenner, the use of plasma, rather than serum, has been tried as a means of shortening the time that must elapse between the taking of a blood sample and the completion of a test. Since plasma can be collected much more rapidly and easily than serum, its use offers the possibility of making a presumptive diagnosis in a few moments after taking of the blood, while the patient waits in the clinic or office.

Plasma specimens were secured by taking blood into tubes containing sodium citrate (final concentration 0.1%). A separate portion of each blood sample was allowed to clot for control tests on the serum. The citrated blood tubes were centrifugalized at once and the plasma collected.

Experiments showed that for the proper performance of the test inactivation of the plasma was necessary. Heating at 56°C. for only 10 minutes was found to be sufficient.

After inactivation the plasma must be recentrifugalized very briefly to remove a heavy amorphous precipitate. The supernatant clear plasma may then be used immediately for the test.

The reaction of the plasma as compared with that of the serum of 212 individuals has been tested by the Kline method. There was an agreement in the reading of the serum and plasma reaction in 76.9% of cases. Of the remaining 23.1% of cases, 6.1% showed minor differences in degree of positivity with a consistently stronger reaction in the plasma; in 9.5% the serum was definitely negative, while the plasma was moderately positive; in the final 7.5% of cases there occurred a doubtful reaction (±) in either serum or plasma with a negative reaction in the other. For all practical purposes the slight discrepancy in the last group may be disregarded; if this were done, the total percentage of agreement between serum and plasma readings would be raised to 84.4%. All the instances in which the plasma gave a definitely positive test, while the serum gave a negative reading, occurred in cases of treated syphilis or in individuals with clinical stigmata of the disease.

Our experience has led us to believe that the marked sensitivity of the plasma reaction may be advantageous in the diagnosis of early syphilis and in instances where the test is employed as a therapeutic guide. However, this same heightened sensitivity may occasionally lead to a doubtful reaction in the citrated plasma of normal individuals. Therefore, in its present form, the Kline test upon the plasma must be regarded as a presumptive, rather than a definitive diagnostic procedure.

By actual trial under the working conditions of our out-patient department, we have found that the total time required to perform the plasma test described, from the taking of the blood sample to the final report, for one specimen, averages about 30 minutes. When blood specimens from a number of patients are to be examined in the routine of clinic practice, it is easily possible for a single worker to handle the plasma specimens nearly as fast as they can be secured, and to complete the tests in an average time of about 5 minutes per test.

Illinois Section.

Michael Reese Hospital, Chicago, April 22, 1930.

4942

Evidence in Support of the Filtration-Reabsorption Theory of Urine Formation in the Dog.

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From the Lasker Foundation for Medical Research and the Department of Medicine, University of Chicago.

In these experiments the amount of urea and glucose excreted in the urine under varying conditions is compared with the amount of similar substance calculated to have been filtered through the glomeruli on the basis of the Filtration-Reabsorption Theory. The amount of urea or glucose filtered is calculated from the following formulae which are adaptations of those proposed by Rehberg[1]:

$$\frac{\text{(Urine Creatinine in mg. per 100 cc.) (Urine Volume in cc. per minute)}}{\text{Plasma Creatinine in mg. per 100 cc.}} = \text{Amount of Glomerular Filtrate in cc. per minute.}$$

$$\frac{\text{(Glomerular Filtrate in cc. per minute) (Plasma urea in mg. per 100 cc.)}}{100} = \text{mg. of Urea Filtered per minute.}$$

Creatinine is assumed to have no threshold value. To make the blood creatinine determinations more reliable creatinine is given by stomach tube 3 hours before samples are collected. All blood concentration figures are averages for the period of observation.

Fifteen experiments have been performed to date.

The amount of urea present in the urine has never exceeded the amount estimated to have been filtered. When urea is injected intravenously an increase in the output of urea is obtained which is approximately proportional to the rise in blood urea concentration except when a spontaneous decrease in the amount of filtrate occurs. Such a decrease counteracts to a large extent the rise in blood urea concentration. Euphyllin administered intravenously increases the amount of urea excreted in the urine and this increase is roughly proportional to increases in the calculated amount of filtrate formed and in the estimated amount of urea filtered.

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[1] Rehberg, P. B., *Biochem. J.*, 1926, xx, 447. *Ibid.*, 1926, xx, 461.

The amount of glucose appearing in the urine after the administration of large doses of phlorhizin is, within the limits of error of the methods employed, equivalent to the amount estimated to have filtered through the glomeruli. When euphyllin is given intravenously the amount of glucose excreted is increased and this increase can be accounted for by the greater amount of filtrate formed. The increase in glucose excretion which occurs when glucose is given intravenously to a phlorhizinized dog corresponds to the increased amount of glucose filtered as a result of the rise in plasma glucose concentration.

TABLE I.
Effect of the intravenous injection of urea upon the excretion of urea in the urine.

Time	Urine Creatinine mg. /100 cc.	Urine Volume cc./min.	Blood Creatinine mg. /100 cc.	Glomerular Filtrate cc./min.	Blood Urea mg. /100 cc.	Urea in Glomerular Filtrate mg./min.	Urea in Urine mg./min.
2.20 to 2.50	3168	0.07	4.72	49.0	8.8	4.3	2.9
251	4.5 gm. urea i.v.						
3.00 to 3.21	320	0.38	4.54	26.8	96.0	26.6	12.6
3.22 to 3.39	510	0.50	4.37	57.7	84.8	48.9	22.1

The excretion of sulphate is now being studied in a similar manner.

The evidence so far obtained would indicate that the process of urine formation in the dog may be accounted for solely on the basis of filtration and reabsorption.

4943

Functional Pathology of the Denervated Kidney.

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From the Department of Pathology, University of Illinois, College of Medicine, Chicago, Illinois, and the Department of Medicine, University of Hamburg, Germany.

Long established clinical observation has made evident the close association of chilling of the body surface with the inception of certain types of nephritis. In those cases where chilling has involved no evident bacterial invasion, this etiological relationship has continued a puzzling and perplexing factor—but one of importance because of the frequent clinical onset under such conditions.

From a series of studies on the leucocytic partition,[1] we have obtained considerable evidence indicating that the autonomic balance of the kidney follows that of the skin, *i. e.,* when the vessels of the skin contract, those of the kidney are likewise contracted. Skin, muscles, brain, kidneys and lungs are apparently associated in this functional grouping of organs—the liver, stomach and spleen being oppositely oriented.

During the course of observations of animals during continuous intravenous injection of *B. coli* extending over long periods of time[2] we noted the effects on the urine. The urine changes give evidence of renal injury (albuminuria, red blood corpuscles and oliguria) which were definitely related to other concurrent systemic changes in the animal.

In further developments of these studies, we have carried out experiments on the denervated kidney. Dogs were used throughout in these experiments and a unilateral denervation carried out under general anesthesia and recovery was then permitted for 2 weeks.

We have previously noted that with bacterial injections there is no evidence of renal injury, as far as urinary changes are concerned, for the period of about 30 minutes after the beginning of the injection—however, with a chill, albumin, red blood corpuscles, etc., appear, later an oliguria and occasionally an anuria. The bacteria appear in the urine very promptly at the time of the chill.

When we now make a continuous injection in animals so prepared, the two kidneys react quite differently. The denervated kidney continues to secrete a normal urine during the course of the entire experiment. No albumin, no red blood corpuscles, no bacteria make their appearance in the urine. The normal kidney, with the onset of the chill, begins to pass albumin, red blood corpuscles and bacteria in large amounts. Later a total anuria may result. The change is clearly evident with the onset of the chill in the animal.

If we take an animal and chill the skin artificially with an ice pack and now begin our bacterial injection, no latent period is necessary before the altered function of the kidney becomes manifest. Red blood corpuscles, albumin and bacteria begin to pass at once into the urine from the kidney that is normally innervated, while the denervated kidney continues to secrete normal urine. The close association of the state of the renal vessels to the possible damage by bacteria and toxins and the obvious importance of the skin-kidney autonomic connection is apparently demonstrated in experiments such as these.

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[1] Müller, E. F., Petersen, W. F., and Hölscher, Rose, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 544.

[2] Petersen, Wm. F., Müller, E. F., and Boikan, Wm., *J. Infect. Dis.*, 1927, xli, 405; Petersen, Wm. F., Milles, George, and Müller, E. F., *Z. fur Gesamte Exp. Med.*, 1927, lx, 336.

4944

The Rôle of Staphylococci in Food Poisoning.

EDWIN O. JORDAN.

From the Department of Hygiene and Bacteriology, University of Chicago.

In food poisoning of the gastrointestinal type attention has been previously focused chiefly on the Salmonella (paratyphoid-enteritidis) group of bacilli. Some European observers have been unwilling to attribute etiological significance to any other organisms. During the past few years, however, material from a number of well defined food poisoning outbreaks, apparently of bacterial origin, has been subjected to thorough bacterial examination in our laboratories without being found to contain any paratyphoid bacilli. I have consequently become convinced that in a considerable number of cases some other bacterial factor is involved. A recent outbreak in Chicago in which a yellow staphylococcus was found by Dack and other workers[1] in this laboratory to be the probable causal agent has led me to carry out some observations on other staphylococcus strains.

With the aid of human volunteers the sterile filtrates of 6 staphylococci have been tested. From 5 to 10 cc. of sterile broth filtrates of these organisms taken by mouth has caused in a few hours the train of food poisoning symptoms commonly met with: dizziness, loss of appetite, nausea, vomiting and diarrhea. These staphylococci are of diverse origin and are not of uniform cultural character. Three of these were isolated from normal human throats, one from a case of septicemia in man and 2 from food implicated in "food poisoning" outbreaks. With the amounts of filtrate used not all volunteers were affected, but out of 34 taking approximately the same quantity 26 became definitely ill with characteristic symptoms.

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[1] Dack, G. M., Cary, W. E., Woolpert, O., and Wiggers, H., *J. Prev. Med.*, 1930, iv, 167.

Eleven control subjects taking the same food at the same time without addition of filtrates remained well, but one reported the occurrence of food poisoning symptoms. Since this individual had been made quite ill with filtrates the previous week, the second attack may have been due to suggestion or to association with those who were ill.

The toxic substance in staphylococcus filtrates that causes food poisoning is destroyed by boiling and either destroyed or greatly weakened by heating at 60-65°C. for 30 minutes.

A second attack has been produced in one and the same individual by feeding a second portion of the same filtrate after a week's interval.

4945

Neutralization of the Virus of Poliomyelitis by Human Sera.*

HOWARD J. SHAUGHNESSY, PAUL H. HARMON AND FRANCIS B. GORDON. (Introduced by Gail Dack.)

From the Department of Hygiene and Bacteriology, University of Chicago.

The investigations of Netter and Levaditi,[1] Anderson and Frost,[2] Peabody, Draper and Dochez,[3] and of Römer[4] among others indicate that the sera of persons who have recovered from an attack of poliomyelitis have the power to neutralize the virus of this disease. Netter and Levaditi[5] and Taylor[6] have shown that sera from this group of persons do not always possess this neutralizing power, however.

The work of Anderson and Frost[2] and of Peabody, Draper and Dochez[3] and of Leake[7] indicates that sera from suspected "abortive" cases of poliomyelitis are not certain in their action against the virus.

These 3 latter groups of observers have reported experiments in which sera of normal persons have sometimes neutralized the virus, although usually they did not.

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* This study was aided by a grant from the Milbank Fund for the Study of Infantile Paralysis of the University of Chicago.

[1] Netter, A., and Levaditi, C., *Compt. rend. Soc. de Biol.*, 1910, lxviii, 617.

[2] Anderson, J. F., and Frost, W. H., *J. Am. Med. Assn.*, 1911, lvi, 663.

[3] Peabody, Draper and Dochez, 1912, *Mono. Rockefeller Institute*, No. 4.

[4] Römer, P. H., *Epidemic Infantile Paralysis*, 1913, Eng. tr., New York.

[5] Netter, A., and Levaditi, C., *Compt. rend. Soc. de Biol.*, 1910, lxviii, 855.

[6] Taylor, H. D., *J. Exp. Med.*, 1919, xxix, 99.

[7] Leake, J. P., *Hygienic Lab. Bull.*, No. 111, 1918.

None of these investigators except Anderson and Frost,[2] who apparently nullified their results by the addition of "normal" human serum to activate the serum-virus mixtures, attempted to study these reactions in a quantitative manner.

In the experiments reported here Berkefeld filtrates of 5% emulsion of spinal cord containing the virus were mixed in 1.5 cc. quantities with an equal quantity of the serum dilutions studied. The resulting mixture was placed in the incubator at 37°C. for 2 hours and then in the ice box for 18 to 20 hours. Two cc. amounts of the mixture were injected into monkeys by the intra-cerebral route. The experiments were planned so that they were controlled by the presence of convalescent sera from monkeys or persons which were expected to neutralize and by sera from infants or normal monkeys not expected to inactivate.

A summary of the principal results is presented in Table I.

TABLE I.

Summary of Neutralization by Sera from the United States and Canada.

Source of Sera	Number of Sera Tested	Number of Sera Which Neutralized in Dilution		
		1:2	1:10	1:30
Persons attacked by poliomyelitis 6 mos. to 2 yrs. before test	7	4	3	2
Persons attacked by poliomyelitis 2 to 10 yrs. before test	2	2	2	2
Persons attacked by poliomyelitis 10 yrs. or more before test	3	2	1	1
Family contacts of poliomyelitis cases	4	4	4	4
Normal persons:				
Under 2 years of age	5	2	0	1?
2-11 years of age	6	5	5	5
12 years of age and over	9	8	8	6

The results indicate that sera of contacts and of normal adults and children possess at least equal and apparently more power to neutralize the virus than the sera of persons who have recovered from an attack of the disease. The sera of infants seems to be almost lacking in effect on the virus.

The results are interesting in their indication that immunization proceeds in the normal population in much the same way as in the case of diphtheria.

Re-tests of 2 of the normal adult sera tested in the ordinary dilutions used have shown that they may neutralize virus in the highest dilution yet tested, *i. e.,* 1 to 200. It is generally assumed, although so far without actual experimental proof, that there is a correlation between the neutralizing power of a serum in the *in vitro* tests and its effectiveness in the treatment of the disease. It would seem worth while in the light of these results for the clinician to study the value of normal sera, known to neutralize virus, in the therapeusis of poliomyelitis. Zingher,[8] using untested normal serum, has already demonstrated the possible usefulness of such measures.

4946

Further Observations on the Pregnancy-Response of the Uterus of the Cat.

R. G. GUSTAVSON AND H. B. VAN DYKE.

From the Department of Physiological Chemistry and Pharmacology, the University of Chicago.

We have previously found[1] that injections of corpus-luteum extract into non-pregnant cats even after ovariectomy change the response of the uteri so that the latter, like uteri of pregnant animals, contract when the hypogastric nerves are stimulated. If such animals are untreated or receive injections of extracts containing only oestrin, their uteri always relax in response to hypogastric nerve-stimulation, thus behaving like non-pregnant uteri.

In a new series of experiments we have injected into cats and kittens sterile urine of pregnant women. The amount injected daily was approximately 10 cc.; the period of injection was about 10 to 14 days prior to a crucial experiment in which we recorded the movements of the uterus *in situ*. Such urine contains oestrin as well as another substance causing precocious sexual maturity with corpus-luteum formation and thought by some to originate in the *pars anterior* of the pituitary body.

Injected into ovariectomized cats, pregnancy-urine caused no change in uterine-response. When such injections were made into unoperated non-pregnant cats or kittens we always observed a marked follicular development, frequently with corpus-luteum formation. Only in animals the ovaries of which contained corpora lutea, did the uteri exhibit a pregnancy-response. This fact we believe constitutes further evidence that the pregnancy-response of the uterus of the cat depends at least in part on a substance elaborated by the corpus luteum.

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[8] Zingher, A., *J. Am. Med. Assn.*, 1917, lxviii, 817.

[1] VanDyke, H. B., and Gustavson, R. G., *J. Pharm. Exp. Ther.*, 1929, xxxvii, 379.

4947

Basal Metabolism and Other Observations Following Oestrin Injections.

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From the University of Chicago.

Preparation and Assay of Female Sex Hormone. The hormone used in this experiment was obtained from human pregnancy urine according to the following procedure:

Urine, made acid to Congo Red, was extracted with butyl alcohol in a continuous extractor for 48 hours. The alcoholic extract was taken to dryness, the residue dissolved in ether and the ether extracted 3 times with a saturated solution of sodium bicarbonate. The ether was removed by distillation and the residue dry-distilled under reduced pressure at 160°C. to remove butyl urethane. The residue was redissolved in ether, cooled to —12°C. and filtered through cotton. The ether solution was taken to dryness and the residue leached out thoroughly with a 5% solution of sodium hydroxide. The alkaline solution was cooled to —12°C. and filtered, acidified till very faintly alkaline to phenolphthalein and extracted 6 times with ether. This solution was assayed and made up for injection by pouring into olive oil and removing the ether by evaporation on a steam bath.

Assay. The method used was the vaginal smear test of Allen and Doisy. For the purpose of this experiment no smear was considered positive unless there was a complete replacement of leucocyte cells by squamous epithelial cells. The unit used is the one regularly employed in these laboratories on routine assays, namely, that amount of material which will bring 3 rats into complete oestrus providing the next higher dosage used does the same for 3 other animals. This preparation assayed as follows:

0.1 mg., E, E, E,; 0.08 mg., E, E, E; 0.06 mg., E, E, E; 0.04 mg., E, E, E; 0.02 mg., E, EL, EL.

Repeat: 0.06 mg., E, E, E; 0.04 mg., E, E, E; 0.02 mg., E, E, E.*

Therefore we consider 0.04 mg. of this material as equal to one rat unit.

Experimental Procedure and Results of Oestrin Injections in Female Dogs. The oestrin prepared and assayed as above was injected subcutaneously into 4 bitches weighing 10 to 12.6 kilos. Two of these were normal and 2 were completely castrated dogs. The daily dose that each dog received was either 100 or 200 rat units. The periods of injection varied from 10 to 39 days. The injections were followed by no signs of untoward effects at the site of injection despite the fact that as many as 39 injections were made in an area approximately 2½ by 1½ inches.

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* "E" signifies a positive reaction, "EL" a mixed smear with epithelial cells predominating, "LE" a mixed smear with leucocytes predominating.

Basal Metabolism determinations were made on 2 of the normal dogs during the period of oestrin injections. One of these dogs received 100 rat units daily for a period of 39 days and the other 200 rat units daily for an equal duration of time. These were dogs belonging to the metabolism series housed in the laboratory for many months and on which many normal basal metabolism tests had been previously made. The variations in the basal metabolism, body temperature, pulse rate, and body weight are easily within the limits of variation which occur under normal conditions. In the dog receiving 200 rat units daily the average basal metabolic rate over the 39 days of injections of the oestrin equalled —2% of the previous normal period. No test either above or below the normal was observed but many low normals (variations of ±5% are considered within the range of normal fluctuation). In the dog receiving the 100 rat units daily the basal metabolic rate is approximately +2 of the normal, a negligible variation. Congestion and enlargement of the external genitalia occurred in all 4 bitches, also a hemorrhagic discharge from the vagina, characteristic of rut in the female dog. Mating behavior became pronounced and copulation was repeatedly observed in both the normal and castrate dogs. Histological examination of the uterus shows marked hypertrophy of the endometrium and evidence of pronounced secretory activity. In one dog (ovaries intact) lactation began on the fifth day of injection and during the following 3 weeks this dog mothered and suckled 2 young, which received no other nourishment. These puppies thrived, apparently, as normal puppies receiving their own mother's milk.

4948

A Forty-eight Hour Test for the Female Hormone with Capon Feathers as Indicator.*

MARY JUHN AND R. G. GUSTAVSON. (Introduced by F. R. Lillie.)

From the Whitman Laboratory of Experimental Zoology and the Department of Physiological Chemistry, the University of Chicago.

We have, as described elsewhere, obtained entirely female plumage in male brown leghorn fowls and capons by injections of female hormone prepared from human placenta, human pregnancy urine and from cow placenta.

The procedure consists in plucking definite areas and injecting the female hormone during the period of replacement which lasts about 3 to 4 weeks. The response of the feather could only be read in about a fortnight or more after plucking as this time is required for the feather germs to regenerate and pierce the sheath.

Since the feather changes are distinct and unmistakable we wished to make use of them as indicators of the female hormone. At the same time it was desirable to obtain readings in a short period of time and with small amounts of hormone. To this end the following method was developed: The breast feathers were used exclusively because their color is black in the male and salmon in the female, offering very distinct contrasts. Furthermore, the feathers in the breast regenerate more rapidly than in other regions of the body.

Breast feathers were plucked in similar regions, at the level of the shoulder, in a number of capons. The feathers were permitted to regenerate for 15 days. At the end of this time the young feathers were about 2.5 cm. in length. Each bird then received 6 cc. of a female hormone preparation containing by assay 100 rat units per cc. distributed in 4 simultaneous subcutaneous injections.

Forty-eight hours after the injections samples of the regenerating feathers were plucked. The sheath was slit, the inner pulp folded back with fine forceps and the feather itself spread out flat against a slide. The female, salmon, pigment deposited during the injection of the female hormone could be seen at once and with the naked eye at the base of the feathers in the majority of test birds. In occasional birds the feathers do not react to a single dosage described above. If the young feathers were plucked 72 hours after the in-

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* The expenses of this investigation were supported in part by the Committee for Research in Problems of Sex of the National Research Council; grant administered by F. R. Lillie.

jection, the female region was yet more distinctly visible as a salmon bar in the black, male feather.

Permanent preparations may be obtained by mounting the feathers in euparal after drying.

Apart from the interest attached to the determination of any new indicator for the female hormone, the method described seems to offer special advantages since it requires the minimum of equipment and errors in reading can scarcely occur.

It is to be hoped as well that the individual variation of the test animals may prove less than in the rat.

Further work is being done on the same line of investigation.

4949

A Synthetic Substitute for Ascitic Fluid in a Medium for Cultivation of Gonococcus.*

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From the Department of Medicine of the University of Chicago.

Ever since its first cultivation by Bumm in 1885, the gonococcus has maintained a reputation for fastidiousness as regards its growth requirements. In consequence, a great number of media have been devised for it, the most successful and reliable of which are those containing human ascitic or hydrocele fluid. It is generally believed that the efficacy of these transudates depends, in large part at least, upon constituents other than their inorganic salts. To determine whether the rôle played by transudates in supporting the growth of gonococci may be attributed to substances other than the biologically important inorganic ions, a study of the function of the individual ions has been undertaken. Although this study is still in progress and will be published in detail later, we wish now to report that it is possible to cultivate the gonococcus on an agar medium containing only beef infusion and dextrose, providing it is made up in an aqueous solution of inorganic salts in kind and concentrations corresponding to those found in an ultrafiltrate of mammalian blood plasma. The solution, which for the sake of brevity we have called van Dyke-Hastings solution, is similar to that used by van Dyke and Hastings[1] in their experiments on the response of guinea pig uterus to pituitary extract and is approximately isohydric, isosmotic, isionic with protein-free transudates. Its composition is given in Table I:

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* This study was made possible by a research grant from the Public Health Institute of Chicago.

TABLE I.

Final Concentration of Inorganic Cations and Anions.

Cation	$\frac{\text{mM}}{\text{Liter}}$	Anion	$\frac{\text{mM}}{\text{Liter}}$
Na^+	145.4	Cl^-	123.6
K^+	6.0	HCO_3^-	30.0
Ca^{++}	1.5	$HPO_4^=$	0.8
Mg^{++}	0.5	$H_2PO_4^-$	0.2

pH = 7.4 CO_2 tension = 46 mm. Hg.

Such a solution is simply prepared by mixing appropriate quantities of isotonic solutions of the individual salts. The composition of the stock solutions to be mixed is given in the second column of figures and the amounts used in preparing the van Dyke-Hastings solution are given in the last column of Table II.

TABLE II.

Salt	Composition of Stock Solutions		Amount of Each Stock Solution Required per Liter Final Solution
	$\frac{\text{mM}}{\text{Liter}}$	$\frac{\text{Grams}}{\text{Liter}}$	
NaCl anhydrous	154	9.0	738.0
KCl "	154	11.47	39.4
$NaHCO_3$ "	154	12.93	195.0
Na_2HPO_4 "	107	15.19	7.6
NaH_2PO_4 "	154	18.48	1.3
$CaCl_2 \cdot 6H_2O$	107	23.45	14.0
$MgCl_2 \cdot 6H_2O$	107	21.77	4.7

It is essential that the calcium and magnesium chlorides be added after the solution containing bicarbonate has been acidified below pH 7.4 with CO_2; otherwise these salts will be precipitated. Ideally the medium should contain enough CO_2 to be in equilibrium with a tension of 46 mm. (*i. e.*, about 6%) of the gas at 37°C. But as the procedure described below was found to yield a satisfactory medium, it proved to be unnecessary to work at this exact tension throughout its preparation.

The medium is made as follows: Fresh beef heart, freed from fat and fibrous tissue, is ground in a meat grinder and added to twice its weight of van Dyke-Hastings solution (*without* Ca and Mg).

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[1] van Dyke, H. B., and Hastings, A. Baird, *Am. J. Physiol.*, 1928, lxxxiii, 563.

The mixture is cooked at 70°C. for 3 hours and then boiled for 15 minutes. It is strained hot through cotton and made up to its original volume with distilled water. One and one-half per cent of powdered agar and 1% of dextrose are added. After they have dissolved, 15 cc. of a 0.02% aqueous solution of phenol red per liter are added to facilitate pH control in the subsequent operations.

The solution at this point is usually alkaline, about pH 7.6. If, as occasionally happens, it is much less alkaline, it can be adjusted to about that reaction with NaOH. It is then put into an Ehrlenmeyer flask fitted with a two-hole rubber stopper through which pass an aeration tube and an outlet tube, each plugged with cotton and the neck wrapped with paper to protect it against subsequent contamination. It is then autoclaved at 15 pounds for 15 minutes. After cooling to about 70°C. CO_2 is bubbled through the medium by means of the aeration tube until the color of the phenol red indicates a reaction of about pH 7.0. At first it may be necessary to withdraw samples of the medium in order to determine its reaction in a comparator block but with a very little experience one is able to estimate from the color of the large bulk of fluid its pH accurately enough for the purpose at hand. At this point the calcium and magnesium solutions, previously sterilized by autoclaving, are added in appropriate amounts. The medium is then tubed as quickly as possible and slanted. After cooling the reaction is usually pH 7.2-7.4, which is quite satisfactory for the growth of the gonococcus. The medium will become alkaline on standing unless kept in an atmosphere of 6% CO_2. During cultivation this is prevented by the fermentation of the dextrose by the growing organisms.

This medium has been found to support the growth of gonococci, both in primary and subculture, quite as luxuriantly as any of the more complex media, or as any of those containing human ascitic fluid.

4950

Cowper's Gland as a Testis Hormone Indicator.*

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Studies on the physiology of the internal secretion of the mammalian testis have been hampered by the lack of standard, easily read, and reliable hormone indicators. Moore,[1, 2] Moore and Gallagher,[3] Moore, Price, and Gallagher,[4] and Moore, Hughes, and Gallagher[5] have used 4 methods of hormone assay on the rat and guinea pig, the spermatozoan motility test, the electric ejaculation test, and the cytological changes that occur in the rat seminal vesicles and prostate gland following castration and also following injections of hormone samples.

In the process of isolating a hormone, there is always the possibility of there being more than one active principle. Therefore it is necessary to study all possible effects of the hormone. This study of Cowper's gland was undertaken to note its relation to the male sex hormone, and to ascertain whether it could be used as an efficient hormone indicator. This problem was suggested by Dr. Carl R. Moore, and is continuing under his guidance.

Over a period of 2 years, 175 white rats and guinea pigs have been utilized. A large number of fixatives have been tried; those most generally used being either Bouin's or Zenker-Formol for histological purposes, and the Mann-Kopsch fixation for the preservation of the Golgi material. Ehrlich's haematoxylin and eosin was the standard stain employed.

Castration of the adult animal has a marked effect on Cowper's gland. In the rat, a noticeable change is seen in 10 days and becomes very marked at 20 days; while in the guinea pig the effect is not clearly seen until about 50 days after the operation. The epithelium of both the rat and guinea pig Cowper's becomes very low.

In the rat, after 20 days castration, a degeneration and merging together of most of the tubules occurs which produces large lumina

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* This investigation has been aided by a grant from the Committee of Research in Sex Problems of the National Research Council; grant administered by F. R. Lillie.

[1] Moore, C. R., *J. Exp. Zool.*, 1928, l, 455.

[2] Moore, C. R., *Biol. Bull.*, 1928, lv, 339.

[3] Moore, C. R., and Gallagher, T. F., *Am. J. Anat.*, 1930, xlv, 39.

[4] Moore, C. R., Price, D., and Gallagher, T. F., *Am. J. Anat.*, 1930, xlv, 71.

[5] Moore, C. R., Hughes, W., and Gallagher, T. F., *Am. J. Anat.*, 1930, xlv, 109.

filled with great masses of secretion. Within 25 to 30 days there is a collapse of these large tubules and an apparent increase in the amount of inter-tubular connective tissue. Also, there is a marked decrease in the gross size of the organ after 60 days castration. The tubules continue to become smaller and merge with one another so that 150 days after the operation only a few small tubules are left, which usually contain a very low epithelium. The whole gland continues to grow smaller so that often they are extremely hard to locate. No degeneration of the striated muscle capsule was noted.

In the guinea pig, castration changes are neither as rapid nor as pronounced as in the rat. The only significant change is about a 50% decrease in height of the cells 120 days after castration.

The Golgi bodies of the epithelial cells of both the rat and guinea pig do not change greatly after castration. There is a tendency for the reticulum to form a cap over the nucleus, but no significant decrease in the amount of osmiophilic material was found. In the guinea pig, no change in either the number or location of the mitochondria was observed.

The Cowper's glands of normal pre-pubertal animals appears much the same as in the normal adult but smaller in size. Castration of young rats causes a degeneration similar to that of the castrated adult animal.

Injections of testis extract, prepared by Mr. T. F. Gallagher of the Department of Physiological Chemistry, have been made, using the rat as a test animal. Adults were castrated and injected for a period of 20 days, at which time the effects of the hormone are readily noted. It was found that a relatively large amount of hormone is necessary to keep the glands in a normal condition. Animals castrated 8 months and then injected for 30 days with an active extract were found to have the normal type of epithelium in the Cowper's glands; although the gland was not as large as the normal. Injections in adults castrated pre-pubertally cause these glands to return to the normal condition.

Cowper's gland of the white rat has proved to be an efficient qualitative testis hormone indicator. It is sufficiently sensitive so that castration effects are easily discernible 20 days after the operation; however, it is not as sensitive to the testis hormone as the spermatozoan motility, or seminal vesicle cytology tests. A more extensive report of this work will appear elsewhere.

4951

Calcium and Phosphorus Content of Epithelial Lined Cysts From Transplantation of Mucosa of Urinary Bladder to Rectus Sheath in Dogs.*

C. B. HUGGINS AND EDWARD L. COMPERE.
(Introduced by Edmund Andrews.)

From the Department of Surgery of the University of Chicago.

In a preliminary report, one of us[1] described the formation of bone around a portion of a cyst lined by transitional epithelium resulting from transplantation of bladder mucosa to certain connective tissue areas (one of which is the sheath of the rectus abdominis muscle) in the dog. Similar transplantation in the rabbit is rarely followed by bone formation. The cysts contain a sero-sanguinous fluid in small amounts, rarely more than 4 cc., usually about 2 cc. when a circular area of bladder mucosa 2 cm. in diameter is transplanted.

It seemed of interest to determine the calcium and phosphorus content and the hydrogen ion concentration of this fluid and to compare these values with similar simultaneous determinations of the blood serum. In 3 rabbits in which bone did not occur around the transplant, determinations were made of the inorganic phosphates and hydrogen ion concentration of the cyst fluid and of the blood serum.

The fluid was aspirated through the skin with an 18 gauge needle and was drawn under oil. Calcium was determined by the Clark and Collip[2] modification of the Kramer and Tisdall[3] method in most instances. In 3 of the determinations the organic material was destroyed by heating with concentrated sulfuric acid before using the above method, with approximately the same results. Phosphorus was determined by the method of Fiske and Subbarow.[4] Carbon dioxide content of the serum and cyst fluid was determined by the manometric method of Van Slyke and Neill.[5] The bicolorimetric method suggested by Myers[6] and Hastings[7] was used for determining the hydrogen ion concentration.

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* This work has been done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Huggins, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 349.

[2] Clark and Collip, *J. Biol. Chem.*, 1925, lxiii, 461.

[3] Kramer and Tisdall, *J. Biol. Chem.*, 1921, xlvii, 475.

[4] Fiske and Subbarow, *J. Biol. Chem.*, 1925, lxvi, 375.

[5] Van Slyke and Neill, *J. Biol. Chem.*, 1924, lxi, 523.

[6] Myers, *J. Biol. Chem.*, 1922, liv, 675. Myers, Schnitz and Booher, *Ibid.*, 1923, lvii, 209.

[7] Hastings and Sendroy, *J. Biol. Chem.*, 1924, lxi, 695.

TABLE I.

Dog No.	Date	Days Transp.	Cyst Fluid					Blood Serum				Ossification in Cyst
			cc. of Fluid	Ca*	P*	CO_2†	pH	Ca*	P*	CO_2†	pH	
256	9-10-29	62	3.0	15.9	11.0		6.32	10.4	5.16	43.16	7.51	++++
212	9-10-29	47	3.6	15.6	9.6		6.45	10.98	3.44	49.2	7.57	++++
215	9-14-29	80	5.4	10.77	8.23		6.35	10.77	4.91		7.43	++++
543	11-18-29	29	2.6	13.35			7.35	10.34			7.42	++++
543	12-18-29	59	10.0	10.72	5.9		6.97	11.07	5.33		7.53	Cyst fluid contains pus
689	2- 3-30	33	2.2			16.4	7.27	11.6	5.3	45.3	7.44	++++
724	2- 3-30	57	5.0			18.3	7.02	10.8	5.71	51.5	7.45	++++
986	2- 3-30	35	9.0	11.04	4.4	28.6	7.17	11.2	5.16	51.3	7.44	++++
821	3-10-30	62	0.5	16.3				11.6	6.15			++++
986	3-10-30	69	0.8	15.2				11.6	6.2			++++

*Milligrams for each 100 cc. †Volume per cent carbon dioxide content.

TABLE II.

Dog No.	Date	Days Transp.	Cyst Fluid					Blood Serum				Ossification in Cyst
			cc. of Fluid	Ca*	P*	CO_2†	pH	Ca*	P*	CO_2†	pH	
928	2- 3-30	100	6.0			114.7	7.69	12.20	6.66	55.9		+ Cyst grossly infected
873 & 506	3-10-30	49 & 62	0.65	17.4				11.86	6.25			++++
212	6-24-29	267	3.0	23.5				10.5				++++
821	4-16-30	113	1.5	18.2	5.22			12.6	6.67			++++
689	4-16-30	118	1.8	16.6	3.0			12.6	6.67			++++
869	4-11-30	67	2.4	16.0	16.8			10.46	6.4			++++
Rabbit No.												
348	12-30-29	50	0.75		7.16		7.32		7.02		7.35	No ossification
353	12-30-29	50	0.6		7.4		7.34		6.7		7.39	No ossification
362	12-30-29	50	1.0		6.7		7.35		7.04		7.34	No ossification

*Milligrams for each 100 cc. †Volume per cent carbon dioxide content.

The results are tabulated in Tables I and II. In 10 of 13 determinations, the calcium was higher in the cyst fluid than in the blood serum. In 5 of 8 determinations of phosphorus, it was higher than in the serum. In 8 of 9 determinations, the cyst fluid was more acid than the blood serum.

In 2 cases the cysts were grossly infected and here the calcium was lower than in the blood serum. In the rabbits, no bone was formed and the hydrogen ion concentration and phosphorus content of the cyst fluid did not differ markedly from similar values in the blood serum.

4952

Effect of Bile Salts on the Blood Calcium.*

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From the Department of Surgery of the University of Chicago.

The relation of calcium metabolism to jaundice has long been a matter of clinical observation. Changes in the bones following both cholemia and prolonged loss of bile are a matter of common knowledge.

More recent work by Walters[1] and Heyd[2] and others has put the therapy of jaundice by the intravenous injection of calcium chloride on a very firm clinical basis so that it is used as a routine in many clinics today. While the effect aimed at has generally been the decrease in the coagulation time of the blood as a preparation for surgical therapy, the results achieved have gone further than that, and it is without a doubt true that there is also a marked temporary detoxification produced by the intravenous injection of large amounts of calcium chloride.

The studies of Still[3] have shown clearly that it is the bile salts which are the offending factor in cholemia as the other elements of the bile are nontoxic.

Buchbinder and Kern[4] have shown that in animals and man the

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Walters, W., *Minnesota Med.*, 1925, vi, 25.

[2] Heyd, C. G., *Am. J. Obst. and Gyn.*, 1930, xix, 203.

[3] Still, E. U., *Am. J. Physiol.*, 1929, lxxxvii, 728.

[4] Buchbinder, W. C., and Kern, R., *Am. J. Physiol.*, 1927, lxxx, 273.

blood calcium tends to be rather low and Cantarow, Dodek and Gordon[5] have confirmed the early work showing a greater excretion of calcium in jaundice. Walters and Bowler[6] show that twice the amount of calcium salt injected intravenously may be required to raise the blood calcium to a given level in a patient with jaundice as in a normal person.

TABLE I.
Effect of Bile Salts on Blood Calcium.

	Control	1 hr.	2 hr.	4 hr.	6 hr.	8 hr.	24 hr.
1. Intraperitoneal, 4 cc. 10% sal. per K	11.2	11.4	11.6	Dead			
2. Intraperitoneal, 2.5 cc. 10% sal. per K	11.6	11.8	11.8	11.6	11.5	11.4	10.7
3. Same	11.1	11.8	11.68	11.36	11.02	10.9	10.58
4. Same	10.69	11.68	10.6	10.36	10.1	10.02	10.0
5. Intravenous (20 min.) 200 mg. per K	12.0	10.6	11.8	11.9	11.58	11.36	10.69
6. Intravenous, 300 mg. per K	11.9	11.8	11.25	11.03	11.03	10.97	10.69
7. Intravenous (20 min.) 300 mg. per K	10.4	10.13	10.13	10.1	9.7	9.6	Dead

With these points in view the following series of experiments were undertaken. A 10% solution of bile salts was injected intraperitoneally into 4 dogs; to 3 it was administered intravenously, and the blood calcium determinations were made at short intervals thereafter. It can be seen from the accompanying table and chart that in each case, exactly the same result is achieved following a short preliminary rise there is invariably a marked fall in the blood calcium. This fact is of interest in its possible relation to the mechanism of the toxemia produced by accumulation of bile salts in the blood. While no explanation of the phenomenon can be given, the obvious fact of the insolubility of the calcium compounds of bile acids suggests their precipitation. Studies of the reticulo-endothelial system are being undertaken to test this hypothesis.

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[5] Cantarow, A., *et. al., Arch. Int. Med.*, 1927, xl, 129.

[6] Walters, W., and Bowler, J. P., *Surg. Gyn. and Obst.*, 1924, lxix, 200.

Iowa Section.

State University of Iowa, April 24, 1930.

4953

Relation of Diet to the Production of Dental Caries in Young Rats.

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The effect of diet on dental integrity has been noted by several observers.[1, 2, 3, 4] Dental caries has been observed to occur in adult rats receiving faulty diets.[5, 6, 7] Desiring to correlate such experiments with the occurrence of dental caries in children, experiments were conducted with young rats, attempting to produce caries through dietary deficiencies.

Rats from an inbred colony were used. Litter mates were assigned to various diets to be employed; the studies were instituted when the rats were 23 to 32 days of age, and continued from 27 to 51 days. Distilled water and food were furnished *ad libitum.* The rats were weighed twice weekly. At the end of the experimental period they were killed and the molars removed for examination. A summary of the results on the male animals will be found in the accompanying table. The female animals reacted in a comparable manner.

Diet D1 consisted of commercial casein, 18%; hydrogenated fat, 20%; Osborne-Mendel salt mixture, 4%; cornstarch, 58%; vitamin B concentrate, 1%.

Diet D2: Diet D1 plus 4% vitamin B concentrate.

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[1] Mellanby, May, *Brit. Dental J.*, 1923, xliv, 1031.

[2] Mellanby, M., Pattison, C. L., Proud, J. W., *Brit. Med. J.*, 1924, ii, 354.

[3] Boyd, J. D., and Drain, C. L., *J. Am. Med. Assn.*, 1928, xc, 1867.

[4] Boyd, J. D., Drain, C. L., Nelson, M. V., *Am. J. Dis. Child.*, 1929, xxxviii, 721.

[5] Marshall, J. A., *J. Am. Dental Assn.*, 1927, xiv, 3.

[6] McCollum, E. V., Simmonds, N., Kinney, E. M., Grieves, C. J., *Johns Hopkins Hosp. Bull.*, 1922, xxxiii, 202.

[7] Bunting, R. W., *Dental Cosmos*, 1925, lxvii, 771.

Diet D3: Diet D1 plus 4% vitamin B concentrate plus 3 drops of cod liver oil daily.

Diet D4: Diet D1 plus 6% vitamin B concentrate plus 3 drops of cod liver oil daily. This was the control diet for this series.

Diet E1 consisted of purified casein, 18%; hydrogenated fat, 20%; Osborne-Mendel salt mixture, 4%; cornstarch, 36%; dried yeast foam, 4%; and dried spinach, 10%.

Diet E2: Diet E1 plus a daily dose of irradiated ergosterol supposedly equivalent to the amount of D given in the cod liver oil in diet D4. This was the control diet for this and the following series.

Diet F1 was identical with E1 except that 2% NaCl was substituted for the Osborne-Mendel salt mixture.

Diet F2: Diet F1 plus a daily dose of irradiated ergosterol as in Diet E2.

TABLE I.

Diet	No. of rats	No. days on diet	Gain gm. per day	Tooth Ca %	Tooth P %
D1	6	28	0.55	—	—
D2	2	47	1.62	—	—
D3	4	49	1.60	—	—
D4	4	42	3.50	—	—
E1	5	51	2.84	27.8	13.7
E2	6	51	4.28	28.5	13.5
F1	4	50	0.76	25.3	11.4
F2	3	50	1.38	23.9	12.5

Caries was not demonstrable in any instance, although the biological incompleteness of the diets was reflected in marked reduction of growth, as compared to the rats on the control diets. Moreover there seems to be a significant difference in the calcium and phosphorus content of the teeth of rats on diets E as compared to those on diets F. The differences between E1, E2 and F1, F2 cannot be regarded as significant.

The failure of these young rats to develop caries is in accord with the results of Marshall,[5] who, working with low A diets, found that while adult rats developed tooth cavities with some regularity, in no instance was there any evidence of carious defects in young rats.

This study does not indicate that dental caries cannot be produced in young rats by diets deficient in some respect. It does indicate that in the young rat dental caries probably does not result from the use of diets low in vitamin A, B or D or by a deficiency of mineral salts.

4954

Relation of Food Consumption of Milk-fed Rats to the Growth of the Suckling Young.

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From the Department of Nutrition, Iowa Child Welfare Research Station, State University of Iowa, Iowa City.

In a recent investigation[1] it was pointed out that the failure of milk fed rats to successfully suckle their young is not due primarily to a deficiency of the antineuritic vitamin in the milk secretion as has been suggested by a number of investigators,[2] but to the inability of the particular rat to ingest enough milk to meet the lactation requirements. In this investigation the mother animals were transferred from the stock ration to the milk diets just previous to or immediately following parturition.

In order to rule out the possible criticism that the results in this previous investigation were vitiated by a carry over of the antineuritic vitamin from the prelactation period when the animals were receiving high vitamin diets, we have fed groups of rats from infancy (4 weeks) both evaporated milk, which has been shown to be lower in the antineuritic vitamin than quickly boiled milk, and dried partially skimmed milk supplemented with butter fat to make it comparable to the evaporated milk. Certain of these groups were given a concentrated alcoholic extract of wheat embryo, calcium phosphate, and viosterol, either singly or together, while others received the milks without these additions. Small amounts of copper, iron, and iodine were added to all diets.

The females in all groups bore young. Shortly after birth the number in the litter was reduced to 6. In some cases the number born was less than 6 or the young died during the first days following parturition, so that there was some variation in the number raised. The food ingestion was measured throughout the lactation period (21 days) and the caloric ingestion evaluated in relation to the body weight of the mother.

All mothers receiving the dried milk during the lactation period gained in weight, whereas those receiving evaporated milk, with one exception, lost slightly. The addition of the wheat embryo extract to the dried milk fed group showed no influence on the food ingestion of the mother, the percentage of weight gain of the mother, or the size of the young. Similarly the addition of the wheat embryo extract to the evaporated milk groups did not influence the food ingestion nor the weight of the mother.

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[1] Daniels, A. L., Jordan, D., and Hutton, M. K., *J. Nutrition,* 1929, ii, 19.

[2] Sure, B., *J. Biol. Chem.,* 1925, lxii, 371; lxiii, 211. Evans, H. M., and Burr, G. O., *J. Biol. Chem.,* 1928, lxxvi, 263.

There seems to be a relation between the amount of food ingested and the gain in weight of the young in each group. The average weekly gain of the young of the animals receiving the dried milk feeding was greater than that of the young of mothers fed the evaporated milk. The caloric ingestion of the former group was slightly greater. Our results with dried milk are comparable to those previously reported. There is no evidence that the prelactation carry over of Vitamin F influenced our previous results to any great extent. The dried milk tested contained enough of the antineuritic vitamin for normal growth, reproduction, and successful suckling of 6 young. Our results with evaporated milk, on the other hand, suggest that some essential constituent may be low, since optimum results were obtained with none of these groups. The results with the Vitamin F additions were slightly better than those without this addition. However, the differences between the animals receiving Vitamin F addition and those not getting it were not sufficiently outstanding for us to conclude that this vitamin is the lacking substance.

4955

Vitamin F in Relation to the Development of the Thyroid and Thymus of Suckling Young.

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When high protein diets, adequate in all the known dietary constituents excepting Vitamin F, are fed to lactating rats, the young, besides manifesting the untoward symptoms previously described,[1] give evidence of abnormal glandular development. The thymus gland is much smaller than that of normal animals of the same age or weight, while the thyroid is much larger. Among our stock fed animals the thyroid was found to average 0.23% of the body weight, whereas the thyroid of the young of the animals receiving high protein diets averages 0.40% of the body weight. When starch free yeast was added to the high protein diet, the thyroid averaged 0.22% of the body weight. When low protein was substituted for high protein in the basal diet, the thyroid averaged 0.28% of the body weight.

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[1] Hartwell, G. A., *Biochem. J.*, 1922, xvi, 825; 1925, xix, 227; Nelson, M. P., *J. Home Econ.*, 1926, xviii, 383; Daniels, A. L., and Brooks, L., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 161; Daniels, A. L., Giddings, M. L., and Jordan, D., *J. Nutrition*, 1929, i, 455; Lawrence, M. R., *Dissertation*, State University of Iowa, 1929.

The young of animals receiving low protein diets to which was added respectively glycin and alanine, amino acids known to stimulate metabolism, developed symptoms comparable to those of the young of rats receiving high protein diets. The influence of these additions on the thymus and thyroid also was similar. The thyroids of the young of the animals receiving glycin averaged 0.40% of the body weight whereas those of the young of the animals receiving alanine averaged 0.30% of the body weight. Glutamic acid, known to be low in its stimulating effect, caused no hypertrophy; the weight of the glands of the young receiving this averaged 0.23% of the body weight.

The average weight of the thymus gland of the young of rats receiving the high protein diet without added antineuritic vitamin was 0.11% of the body weight. When Vitamin F was added the thymus averaged 0.43% of the body weight.

The addition of glycin and alanine to the low protein diets resulted in an atrophied thymus (0.12% of the body weight). With Vitamin F additions the glands averaged 0.42% of the body weight. The results with glutamic acid, on the other hand, compared with those of the low protein diets.

4956

Influence on Development of Suckling Young of Addition of Certain Amino Acids to Diet of Mother During Lactation.

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From the Department of Nutrition, Iowa Child Welfare Research Station, State University of Iowa, Iowa City.

In studies pertaining to the influence of the diet of the mother on the development of the suckling young of rats, it was shown that a high percentage of protein and inadequate amounts of Vitamin F fed during the lactation period result in certain untoward symptoms in the young.[1] These symptoms were made manifest by extreme nervousness, muscular incoordination, sharp cries, and violent convulsions. In many cases, death followed.

In order to determine the cause of these abnormal conditions in the suckling young, lactating rats were fed a series of diets in which the amount of protein as well as the kind of protein differed. The amount of the antineuritic vitamin at the different protein levels also was varied. In some cases the Vitamin F containing substance was autoclaved at a definite pH to rule out the possible influence of other substances contained therein. Other groups of animals received low protein diets to which were added certain amino acids: glycin and alanine known to have a stimulating effect on the metabolic process, and glutamic acid, which has been shown to have no influence. The effect of Vitamin F additions with these amino acid diets also was tested.

The untoward symptoms in young of the animals receiving the low protein diets with the addition of glycin and alanine respectively were comparable to those of the young of mothers receiving high protein diets; whereas the young of the animals receiving the low protein diets with glutamic acid additions developed normally, as did the young of animals receiving the low protein diets with glycin to which yeast was added.

From these results it seems probable that the need for more antineuritic vitamin when high protein diets are fed is due to the increased metabolism resulting from the action of certain of the amino acids contained therein.

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[1] Hartwell, G. A., *Biochem. J.*, 1922, xvi, 825; 1925, xix, 227; Nelson, M. P., *J. Home Econ.*, 1926, xviii, 383; Daniels, A. L., and Brooks, L., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 161; Daniels, A. L., Giddings, M. L., and Jordan, D., *J. Nutrition,* 1929, i, 455; Lawrence, M. R., *Dissertation,* State University of Iowa, 1929.

4957

Sex Development in Parabiotic Chains of the California Newt.*

E. WITSCHI.

From the Zoological Laboratory, State University of Iowa.

In a previous communication[1] it has been shown that in *parabiotic twins* of *Triturus torosus* the heterosexual combinations give evidence of an intense antagonism resulting in the decrease of the number of germ cells in both testes and ovaries. In some cases the ovaries were found completely sterile and often they showed a tendency toward sex reversal *(free-martin effect)*.

In an attempt to modify this experiment in its quantitative component, *parabiotic chains* have been made by grafting one embryo with its front end on to the tail bud of another embryo. Similar chains of 3 and 4 individuals lived up to 2 months, though only chains of 2 developed and metamorphosed at the same pace as the single controls. About 50 of these chains have been raised. A few, now over one year old, are still alive. Over half of them have been cut in serial sections.

There is some variation in the mode of union of these chains. As a rule the first animal ends at the pelvic region, where the second one is broadly attached with the midbrain region of its head. The spinal cord of the first and the brain of the second animal are grown together in most cases. Despite this very intimate fusion the larger bloodvessels have never been found connected. The two circulatory systems are linked through capillaries only.

The sexes, as expected, are found in 4 combinations. (1) In case both animals are females, the first one has without exception, the better developed ovaries, with larger ovocytes. (2) If both are males, a corresponding difference in size is even more pronounced. One may suspect this difference as being due to better nutritional conditions in the first animal. However, the fat bodies of the second one are well developed, in fact often oversized, while in starved controls they become reduced sooner than the gonads. It is more likely that the slow gonadal development of the second animal is a consequence of the absence of the hypophysis, which, at the time of operation has been partly or completely removed, together with the anterior end of the head. Insufficiency of the hypophyseal hormones is clearly expressed in the light color of most of the posterior animals. That they are not entirely lacking is evidenced, on the other hand, by the growth of the thyroid and the normal progress of metamorphosis. Control experiments show that hypophysectomy in 4 month old larvae results in a distinct underdevelopment of the testes after only a few months, while the ovaries are little or not at all retarded during the first year.

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[1] Witschi, E., and McCurdy, H. M., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 655.

(3) Chains composed of an anterior male and a posterior female, show a nearly normal condition of the testes while the ovaries are always very rudimentary and in most cases completely sterile.

(4) If, on the contrary, the first animal is a female and the second one a male, one finds more varied results. In most cases the female has well developed ovaries with large ovocytes while the testes of the male are rudimentary. In a few cases both ovaries and testes are poorly developed, duplicating thus the status generally found in the parabiotic twins. In one case the testes of the hind animal are much stronger developed than the nearly sterile ovaries of its frontal parabiont.

It is evident that the sexual antagonism observed in male-female parabiosis of the newt *Triturus torosus* as a rule attains a lesser expression if the animals are arranged in chains than if grafted side by side like Siamese twins. This difference is largely due to the slower development of the sex glands of the posterior member of the chains, but partly also to a less efficient transfusion of hormones in this combination.

4958

Some Observations on Ferric Chloride Addition to the Diet.

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From the Department of Chemistry, Iowa State College, Ames.

A specific vitamin for reproduction is well recognized at the present time. This investigation forms a part of a general study of the effect on metabolism of diets lacking, or extremely deficient, in vitamin E.

Waddell and Steenbock[1] state that vitamin E may be destroyed very completely by the addition of 1% of ferric chloride to the diet.

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[1] Waddell, J., and Steenbock, H., *J. Biol. Chem.*, 1928, lxxx, 431.

Vitamin A was not destroyed by this treatment nor, apparently, did the ferric chloride have any other detrimental effect.

We have found that growth and reproduction are normal on a ration of casein 18.0, salt mixture 185, 3.7, cod liver oil (Squibb) 5.0, wheat embryo 12.0, and dextrin 61.3. Rats remain fertile on this ration for at least 2 years. When 1% of ferric chloride is added to this ration growth is not normal. The results on growth depend on how the ferric chloride is added to the ration. There is practically no gain in weight from the beginning if added in powdered form. Half of the rats were dead at 6 weeks. Many of the animals showed sore eyes suggestive of destruction of vitamin A. When the ferric chloride is added to the ration by means of an ether solution, some growth occurs, but it is decidedly below normal.

Waddell and Steenbock's ration consists of the following: yellow corn 71.5, linseed oil meal 15.0, crude casein 5.0, alfalfa meal 2.0, butter fat 5.0, bone ash 1.0, and sodium chloride 0.5. To 5 parts of this mixture was added 1 part of whole milk powder. When ferric chloride in ether solution was added to this ration so as to give a concentration of 1% of ferric chloride, growth again was decidedly below the normal. The normal or control results were obtained on the same ration without ferric chloride.

We next used a ration in which cod liver oil had been treated with ferric chloride. Twenty grams of ferric chloride dissolved in about one liter of ether were added to 100 gm. of cod liver oil. This mixture was placed on 2000 gm. of dextrin in an open vessel and allowed to stand for 3 or 4 days. The mixture of cod liver oil and ferric chloride was then extracted from the dextrin with anhydrous ether for 24 hours. The ferric chloride was removed from the ether solution by repeated washing with distilled water in a separatory funnel until the wash water gave no reaction for iron by the thiocyanate or ferrocyanide tests. The ether was evaporated from the oil which latter possessed a dark color and a slight abnormal odor. Five parts of this oil were added to casein 18.0, salts 185, 3.7, extracted wheat embryo 12.0, and dextrin 61.3. Growth was fair but considerably below the normal. The normal results were obtained on the same ration containing cod liver oil treated as above except that no ferric chloride was added.

Wheat embryo oil was treated with ferric chloride in the same manner as the cod liver oil except that 60 gm. of wheat embryo oil were used in place of 100 gm. of cod liver oil. Three parts of this oil were added to casein 18.0, salts 185, 3.7, extracted wheat embryo 12.0, butter fat 5.0, and dextrin 58.3. Growth was very poor compared with the control. Several rats had inflamed and swollen eyes. The condition of the eyes improved with the feeding of a fresh batch of the ration. The wheat embryo oil after washing still contained 0.3% of Fe. The controls received wheat embryo oil which had an identical treatment except that no ferric chloride was added.

Bakers analyzed C. P. ferric chloride was used. Rats of 50 to 55 gm. weight were employed in all of the experiments.

The results indicate either a destruction of some unknown substance, other than vitamin E, which is necessary for normal metabolism, toxicity of $FeCl_3$—or products produced by it—destruction of some of the known components of the ration, or that ferric chloride completely destroys vitamin E; and that this latter vitamin must be classed as a growth-promoting vitamin, but only very small amounts are needed for normal growth.

4959

The Effect of Experimental Hyperphosphatemia on Calcium and Phosphorus Excretion.

J. D. BOYD, H. M. HINES AND GENEVIEVE STEARNS.

From the Departments of Pediatrics and Physiology, State University of Iowa, Iowa City.

Abnormal levels of serum phosphorus are found in association with various metabolic disturbances, such as renal insufficiency, hyper- and hypo-parathyroidism, rickets and osteomalacia. In this study, we have attempted to determine the effect of experimental hyperphosphatemia upon some of the other constituents of the serum, and upon the urinary constituents. The work was planned to supply values which could be used as standards of comparison for future studies, using animals that will be subjected to various procedures which might be expected to alter their mineral metabolism.

Sodium glycerophosphate injections were used to raise the serum phosphate level. The glycerophosphate was chosen because it was thought that it would be tolerated in larger amounts than the inorganic salt, and because of the growing opinion that phosphate esters may play an important rôle in the metabolism of calcium and phosphorus.

Dogs were given intravenous injections of 50% solution of sodium glycerophosphate, in amounts equivalent to 0.1 gm., 0.25 gm., 0.5 gm., and 1.0 gm. per kilo of body weight. The preparation contained 11% of phosphorus, and gave no test for inorganic phosphate. In control experiments on the same animals, phosphate was given as the sodium salt, pH 7.4, in amount equivalent to 0.5 gm. of sodium glycerophosphate per kilo. The animals were catheterized before, ½ hour and 1 hour after injection. The pH of the urine was estimated, and the specimens were analyzed for calcium, and for inorganic and total phosphorus. The serum studies included the determination of alkali reserve, chloride, calcium and inorganic phosphorus. Nine animals were used; 76 injections were made in all. The earlier determinations served to establish the normal resting excretion of calcium and phosphorus, and the duration of the post-injection period which was significant for study. The data presented in the accompanying table are the average values of 36 injections.

TABLE I.

Dosage mg. P per kg.	Average Excretion During ½ Hour and 1 Hour After Injection.						
	Calcium		Total Phosphorus % of total injection			Organic Phosphorus % total excretion	
	1st ½ hr. (mg./kg.)	Total 1st hr. (mg./kg.)	1st ½ hr.	Total 1 hr.	2nd ½ h. / 1st ½ h.	1st ½ hr.	Total 1st hr.
Sodium Glycerophosphate:							
11	.028	.032	37.5	57.5	53.6	20.4	13.1
27.5	.045	.045	27.0	39.4	45.7	52.5	43.5
55	.229	.274	26.3	39.3	42.0	67.5	54.6
110	1.050	1.364	35.9	52.6	50.7	87.7	72.5
Buffered Sodium Phosphate:							
55	.197	.286	34.3	53.7	56.6	—	—
Normal Resting Excretion:							
none	—	.042	—	.48 mg.	—	—	—

It will be noted that regardless of the size of the dose, from 40 to 60% of the injected phosphorus was eliminated within an hour after injection. Two-thirds of this was eliminated during the first half hour. Even with the smaller doses, part of this was excreted as an organic compound; the amount so eliminated increased with the dosage, most of it appearing in the organic form when the dose was large. The organic excretion was constantly greater during the first half hour than during the remainder of the hour.

There was a definite increase in urinary calcium coincident with the phosphate excretion. The elimination of calcium was roughly proportional to the amount of phosphorus administered, no matter whether given as the organic or inorganic phosphate. With the smaller doses the amount of calcium excreted was so small that the significance of the results in those analyses is open to question. The values were greater, however, than the normal resting values. With larger doses of glycerophosphate, the calcium excretion was greatly increased.

With the larger doses, the serum calcium was definitely depressed. Evidence of moderate or severe tetany was noted when inorganic phosphate equivalent to 0.5 gm. sodium glycerophosphate per kilo was given, yet twice this amount of the sodium glycerophosphate could be given before similar symptoms were evoked. The alkali reserve and serum chloride values showed no significant deviations from normal levels.

Minnesota Section.

University of Minnesota, April 30, 1930.

4960

Glycogen Formation in Rats. III. Diets Containing 87.5 Per Cent of Total Caloric Value in Lactose, Glucose and Sucrose.*

ESTHER M. GREISHEIMER AND OLGA H. JOHNSON.

From the Department of Physiology, the University of Minnesota.

Each diet contained 87.5 gm. of the carbohydrate, 14.7 gm. of casein (85% casein) and 4 gm. of salt mixture 185. This gave a caloric value of 3.77 cal. per gm., of which 87.5% was derived from carbohydrate and 12.5% from protein (not considering digestibility). Each diet was supplemented by vitamin feeding daily.[1]

Adult rats which had served for breeding purposes until discarded, were used. The time on the test diet varied from 16 to 18 days. The food and water intake was measured. The technique followed at the end of the test period was similar to that previously described.[2]

The rats fed on the lactose diet developed a diarrhea. They disliked the diet as evidenced by their scratching it from the food box, consequently we could not measure the intake accurately. As nearly as we could tell, the average daily intake was 7.9 gm., and the intake during the last 24 hours was about the same. The daily intake on the glucose diet averaged 13.3 gm. with 14.4 gm. as the last 24-hour intake. The sucrose daily intake averaged 14.3 gm., with 14.0 as the average for the last 24 hours.

The water intake on the lactose diet averaged 27 cc. per day, influenced, of course, by the diarrhea. On the glucose diet it was 13.9 cc., and on the sucrose diet 10.8 cc.

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* A grant from the Medical Research Fund helped to defray part of the cost of this study.

[1] Greisheimer, Esther M., and Johnson, Olga H., *Am. J. Physiol.*, in press.

[2] Greisheimer, Esther M., and Johnson, Olga H., *Am. J. Physiol.*, 1929, xc, 369.

The mean body weights are given for the beginning and end of the test period:

Lactose (6 females, 4 males) 283.9 gm.—246.3—loss 37.6 gm.
Glucose (6 females, 4 males) 311.6 gm.—314.2—gain 2.6 gm.
Sucrose (3 females, 2 males) 307.8 gm.—318.7—gain 10.9 gm.

The liver glycogen averaged 3.15 ± 0.12% on the lactose diet; 4.89 ± 0.21 on the glucose, and 4.89 ± 0.20 on the sucrose. Expressing the glycogen content as milligrams per gram of body weight gave 0.936 ± 0.057, 1.534 ± 0.066, and 1.764 ± 0.088 mgm., respectively. The glycogen is significantly lower on the lactose diet.

The mean absolute liver weights were 7.04 ± 0.25 gm. on the lactose diet, 9.84 ± 0.36 on the glucose, and 11.13 ± 0.70 on the sucrose. Expressing the liver weight as per cent of body weight gave 2.94 ± 0.11, 3.15 ± 0.07, and 3.62 ± 0.13, respectively.

The calorie intake per gram of body weight during the last 24 hours was determined. This was 0.1209 for lactose, 0.1728 for glucose, and 0.1656 for sucrose. If the liver glycogen, expressed as milligrams of glycogen per gram of liver, be divided by the calorie intake, we find that for each cal. (per gm. of body weight) intake on the lactose diet there are formed 260 mgm. of glycogen per gm. of liver. On the glucose diet the figure is 283, and on the sucrose 295. These figures suggest that if lactose were as readily digested and absorbed as the other sugars, we should find that glycogen is formed with equal ease from sucrose, glucose and lactose.

4961

Glycogen Formation in Rats. IV. Diet Containing 87.5 Per Cent of Total Caloric Value in Maltose.

ESTHER M. GREISHEIMER AND OLGA H. JOHNSON.

From the Department of Physiology, the University of Minnesota.

Fifteen healthy young male rats, varying from 145 to 226 gm. in weight, were given us by Dr. Palmer of the University Farm. They had been on a normal diet, and had not been subjected to experimental procedure. We placed 5 of them on McCollum's Stock Diet I, for controls. The remaining 10 were placed on a diet containing 87.5 gm. of maltose, 14.7 gm. casein (85% casein), and 4 gm. of salt mixture 185. This diet had a caloric value of 3.77 per gm., of which 87.5% was derived from maltose and 12.5%

from casein, not considering digestibility. The analysis and caloric value of the stock diet was given in a previous publication.[1] The Maltose diet was supplemented by vitamin feeding daily.[2] The experiment was in progress for 16 days.

Both the control and the experimental rats gained in weight. The former averaged 3.4 gm. per day, the latter 3.2 gm. The average daily intake of food was 14.13 gm. on the stock, and 14.19 gm. on the maltose diet. The daily water intake averaged 20.76 cc. on the stock, and 15.91 cc. on the maltose diet.

The following results were found:

TABLE I.

	Liver Weight gm.	Liver as % of Body Wt.	% of Liver Glycogen	Mgm. Liver Glycogen per Gm. Body Wt.
Stock diet	10.08 ± 0.11	4.37 ± 0.17	4.86 ± 0.14	2.129 ± 0.075
Maltose diet	10.50 ± 0.18	4.50 ± 0.20	5.93 ± 0.24	2.686 ± 0.132

The difference in liver weight is not statistically significant. The liver glycogen formed on the maltose diet is significantly higher than that formed on the stock diet.

If we consider the last 24-hour intake in terms of calories per gram of body weight, we find 0.2785 for the stock, and 0.2854 for the maltose diet. If the liver glycogen, expressed in terms of milligrams per gram of liver, be divided by the caloric intake, the quotient is 175 for the stock and 208 for the maltose rats. The ability to form glycogen is greater on the maltose diet, as would be expected.

4962

Direct Vestibulo-Spinal Fibers in the Medial Longitudinal Fasciculus of the Cat.*

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In spite of a considerable amount of experimental work on the fiber connections of the vestibular nuclei, there are still some points on which there is disagreement. The marked disturbance in posture and eye movements resulting from vestibular lesions makes it highly desirable to have a very accurate knowledge of the nervous circuits arising from the various vestibular nuclei. To contribute further data on several of the disputed questions, we have produced mechanical lesions in various parts of these nuclei in 20 cats. The cat was used not only because the vestibular system is well developed in this animal, but also because in the course of various other degenerations experiments carried out in this laboratory on the cat, the vestibular nuclei have been unintentionally damaged, thus incidentally contributing data on the vestibular system.

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[1] Greisheimer, Esther M., and Johnson, Olga H., *J. Nutrition,* in press.

[2] Greisheimer, Esther M., and Johnson, Olga H., *Am. J. Physiol.,* in press.

* Aided by a grant from the Medical Research Fund of the Graduate School, University of Minnesota.

After allowing 2 weeks for degeneration, the brains were kept 3 weeks in 3% potassium dichromate, changing the fluid daily during the first week. Blocks 2 to 3 mm. thick were then kept for 10 days in a relatively large quantity of 3% potassium dichromate containing not over 1/3% osmic acid. Most of the disturbing artifacts are eliminated by thus decreasing the duration in the osmic acid solution and reducing the osmic acid concentration.

In most particulars our series confirm the specific conclusions of Gray[1]: that the superior vestibular nucleus gives origin to fibers which ascend in the medial longitudinal fasciculus of the same side, that the medial (triangular) nucleus sends both ascending and descending fibers into the medial longitudinal fasciculus of the opposite side, and, of course, that the lateral (Deiters') nucleus is the origin of the well known descending lateral vestibulo-spinal tract. In some other particulars, however, we are forced to make somewhat different deductions particularly on the question of direct descending vestibular fibers in the medial longitudinal fasciculus, which Gray was unable to demonstrate.

Extensive unilateral lesions in the region of the medial portion of the vestibular nuclei may result in much descending degeneration in the medial longitudinal fasciculus of both sides; but it appears from the work of others that much of this is due to destruction of cells in the reticular formation outside of the limits of the vestibular nuclei. However, we have succeeded in placing a small lesion in the lower portion of the medial vestibular nucleus (where it is prolonged downwards as the descending nucleus) with slight involvement of the lower pole of the lateral vestibular nucleus. The lesion was made by passing a hypodermic needle, containing a concealed knife, through the cerebellum, caudal to the dentate nucleus, entering the vestibular nuclei just below the stria medullaris acoustica. There being no degeneration in these stria nor in the corpus trapezoideum, shows that the cochlear nucleus was not damaged. Our own work, as well as that of others, show that the passage of the needle through the cerebellum could not be responsible for any of the degeneration in the medial longitudinal fasciculus. Even extensive cerebellar injuries produce no degeneration in this tract. Yet with lesions at the point described and slightly lower but well within the limits of the vestibular nuclei and certainly not directly involving the reticular formation, as generally mapped out in atlases, there may be as much or even more descending degeneration in the medial longitudinal fasciculus of the same side as in the one on the opposite side. The direct fibers are situated more dorsally and laterally than the crossed fibers in the cross-section area of these fasciculi. These fibers extend well into the spinal cord. Since there is no descending degeneration in the medial longitudinal fasciculus after lesions strictly limited to the lateral vestibular nucleus, we conclude that the medial vestibular nucleus or its continuation, the descending nucleus, give origin both to crossed and direct descending spinal fibers via the medial longitudinal fasciculus. Lesions too high in the medial nucleus produce crossed degeneration only.

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[1] Gray, L. P., *J. Comp. Neur.*, 1926, xli, 319.

The only possibility of misinterpretation that we can think of is in connection with the blood supply to the adjacent reticular formation, which might have been interfered with sufficiently by the lesion to have caused degeneration of cells outside of the vestibular nuclei, although there is no evidence of such vascular disturbance.

4963

Sugar in 0.02 cc. Blood by the Method of Folin and Malmros.

J. F. MC CLENDON.

From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis.

The method of Folin and Malmros[1] permits of drawing a second sample of the protein-free blood centrifugate in order to duplicate the determination. In the training of assistants, I have taken advantage of this in order to check their technique but in no case was an absolute check obtained. On trying it myself I did not get an absolute check. The reason for this discrepancy may be that sufficient time was not allowed for a diffusion-equilibrium of the sugar between the precipitate and the centrifugate with consequent continuation of the diffusion during the centrifuging and difference in the concentration of the upper and lower part of the centrifuge tube. Another possible cause for the variation is the destruction of sugar in the centrifugate with time, as some of these checks were made 6 or more hours later. Another source of error is in the time taken for making the readings. The 2 solutions of prussian blue, 1 of the blood centrifugate and the other of the standard sugar solution, change on standing and the rate of change is not the same in the 2 solutions. Therefore, one must work rapidly in making the readings. Since the above method of check does not check the technique of measuring the blood sample and since 2 samples of 0.1 cc. of blood can not be taken out of the same drop from the cut finger and since successive drops may vary in sugar content, we have reduced the quantity to one-fifth. Our technique is as follows: using B. & L. apparatus, including the blood pipette made for the hemoglobin determination:

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[1] Folin and Malmros, *J. Biol. Chem.*, 1929, lxxxiii, 115.

Measure 0.02 cc. of blood and blow it into 2 cc. of tungstic acid solution contained in a 5 cc. test-tube. Rinse the pipette by sucking and blowing and keep the mixture stirred for one whole minute and centrifuge until clear. Transfer 1 cc. of the clear fluid with a dry pipette to a second tube, graduated at 5 cc. Rinse the same pipette with the standard sugar solution and transfer 1 cc. of the standard to another tube graduated at 5 cc. To the standard and unknown add 0.4 cc. of potassium-ferricyanide solution and 0.2 cc. of cyanide-carbonate solution. Heat to boiling for 8 min., cool in running water 1 min., add 1 cc. of ferric iron-ghatti solution, shake, and let stand 1 min. Then fill to the mark with H_2O, invert each tube once and pour the total of each tube into a dry flare-top cup of a "biological" colorimeter, placing the unknown on the left and set at 20 mm. Using daylight passed through a jar of potassium ferricyanide solution, make 10 readings as rapidly as possible.

The question arises whether one is able to measure as accurately in this apparatus as in the Folin apparatus, which is 5 times as large. It is a little difficult to check one method against the other unless 2 persons work at the same time, one with one method and one with the other method, owing to errors due to letting solutions stand. (The apparatus was calibrated by weighing water from the delivery pipettes in small weighing bottles and weighing the blood pipettes dry and then filled to the mark with water and dividing the difference in weight by 0.997. The 5 cc. graduated tubes were calibrated by means of a 5 cc. certified pipette.) By working rapidly it was possible to check the method of using 0.02 cc. with that using 0.1 cc. of blood, the former reading between 99 and 101% of the latter. We have found that the blood pipettes fill better if rinsed with distilled water and dried in an oven or by sucking air through, than by drying with alcohol and ether. This may be due to a film of adsorbed water on the pipettes rinsed last in water, or to a film of oil due to the alcohol and ether not being absolutely pure. The ferricyanide used as a color filter must be kept covered with an opaque box when not in use.

4964

Studies of Skin Protection to Ultraviolet Light by Previous Irradiations.

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From the Laboratory of Biophysics, University of Minnesota.

The amount of radiation from a mercury quartz arc required to produce an erythema of a certain skin area of a certain individual is, as a rule, greater the more this area has been exposed to ultraviolet light of wavelength 240 to 310 mμ (the region responsible for the reaction) during the previous 2 months. Evidently some change has been produced in the skin by the first irradiation which makes it less sensitive to the following exposure. What change is responsible for this protection is not definitely known, but it has been shown that it is a local change extending over the exposed area and slightly beyond the margin. It has also been proven that it does not run parallel to the tanning which is another effect produced by certain ultraviolet light bands between 240 and 370 mμ.[1]

For treatments with ultraviolet light it is important to know when and to what extent such protection is produced. Some experiments along this line have been carried out by other investigators and we have attempted to check their measurements and to contribute some further details.[2]

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[1] Uhlmann, E., *Strahlentherapie*, 1930, xxxv, 361.

[2] Schall, L., and Alius, H. J., *Strah.*, 1928, xxvii, 769. Linser, K., and Kropatch, A., *Strah.*, 1926, xxii, 514. Perthes, G., *Munchen Med. Wchschr.*, 1924, lxxi, 1301. Keller, P., *Strah.*, 1924, xvi,; 1928, xxviii, 152.

The development and the consequent progress of the protection varies in different individuals, but the general characteristics of the course seem to be the same for most persons. Some exceptions have been noticed and this fact must be kept in mind as such exceptions are apt to be more common among patients than among healthy individuals.

We have experimented with 3 healthy individuals and the main procedure has been to irradiate one fair-sized area and then to repeat the irradiation at different times to different parts of this region. A new Victor mercury arc lamp was used without any filter. The current was 4.5 amperes at 65 volts and the distance from burner to skin 30 inches.

Experiment I showed that protection was obtained after 7 days against 3 minutes on area originally irradiated 3 minutes and for 4 minutes or more on the same area on the following day. It is interesting to note that the protection was complete enough for 4 more minutes on the 8th day in addition to the 3 minute dose on the 7th day.

Experiment II showed protection after 7 days against 4 minutes on area originally irradiated 4 minutes.

Experiments III and IV showed protection after 6 days against 3 minutes on area originally irradiated 3 minutes.

The results so far obtained can be summed up as follows: (1) A difference in sensitivity to ultraviolet light was noticed on the same individual even on adjacent areas on the anterior surface of the thigh. (2) Two equal exposures produced more reaction when administered some hours or even days apart than when given in immediate succession. (3) An interval of 7 days between successive exposures resulted in protection in all 4 experiments, while an interval of 6 days proved adequate in 2 instances and inadequate in the remaining 2. (4) These observations are in fair agreement with previous works, but the quantitative aspect of the protection has been more clearly brought out.

4965

Effect of Posterior Pituitary Lobe Extracts on the Intestine of Man and Animals.

H. A. CARLSON. (Introduced by O. H. Wangensteen.)

From the Department of Surgery, University of Minnesota.

The stimulating action of pituitary extract on the intestine of man has been widely accepted on the basis of clinical observation. However, experimental studies employing different preparations, different physiological methods and different animals have yielded inconstant results.

Bell and Hick[1] were the first to report a stimulating effect on the intestine of rabbits. McDonald[2] and his co-workers failed to produce defecation in cats after repeated doses and recently McIntosh and Owings[3] reported relaxation of the intestine of the dog after injection of pituitary preparations.

Studies of the effect of pituitary extract on the intestine of man have been made by Pancoast and Hopkins[4] who with the X-ray method found no definite evidence of acceleration of a contrast medium through the bowel. On the other hand, Kümmel, Jr.,[5] with the abdomen of patients open noted that injections of pituitary extracts caused intestinal contractions followed by a period of active peristalsis.

The present study was begun on dogs with intestinal obstruction but later normal dogs were included. The first experiments were conducted on anesthetized dogs but subsequently a series of unanesthetized animals with Thiry-Vella fistulas was added for comparison. Tracings were made by means of balloons inserted into the intestine. In all, 23 experiments were performed on the dog. Cats and rabbits were studied briefly, 2 experiments being made on each. In order to study the effect on man 5 patients with colostomies and one with an ileostomy were selected, tracings being taken to record the intestinal movements.

In a series of 11 dogs under ether anesthesia to which pituitary extract was administered subcutaneously, or intravenously in doses of 0.5 to 2 cc. relaxation or decreased activity of the intestine was observed 6 times. (Fig. 1); no significant response occurred 4

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[1] Bell, W. B., and Hick, P., *Brit. Med. J.*, 1909, February and March.

[2] McDonald, A. D., *Quart. J. Exp. Physiol.*, 1925, xv, 191.

[3] McIntosh, C. A., and Owings, J. C., *Arch. Surg.*, 1928, xvii, 996.

[4] Pancoast, H. K., and Hopkins, A. H., *N. Y. Med. J.*, 1917, cv, 289.

[5] Quoted by Schmidt, H., *Zentralbl. f. Chir.*, 1925, lii, 121.

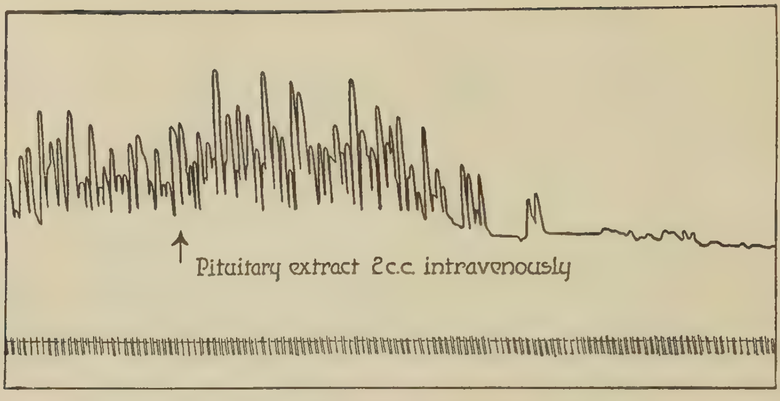

FIG. 1.—Normal bowel, anesthetized dog.

times and in only one instance was there evidence of intestinal stimulation. In 2 further experiments with the vasopressor principle (pitressin) given intravenously, no definite effect on the bowel was noted.

In a series of 5 unanesthetized dogs, no significant changes were observed in 3 cases following the injection of the extract. In one case there was a period of temporary relaxation followed by a moderate increase of tone and activity, and in another there was a slowing of the movements associated with an increase in the strength of the contractions (Fig. 2). In 5 similar experiments in which pitressin was used, the results were almost identical, there being no response 3 times and evidence of intestinal stimulation twice. The phenomenon of decreased activity and relaxation of the intestine was not observed in the unanesthetized dog.

In 2 experiments on the cat no definite intestinal response occurred but in 2 rabbits definite increase of peristalsis following intravenous administration of the pituitary extract was noted.

In 3 patients with colostomies to whom 1 to 2 cc. of pituitary ex-

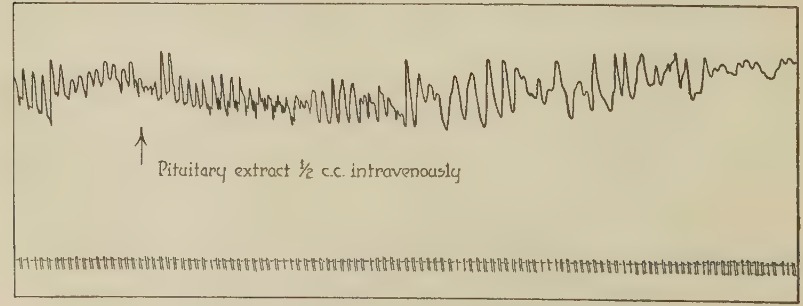

FIG. 2.—Dog with Thiry-Vella fistula.

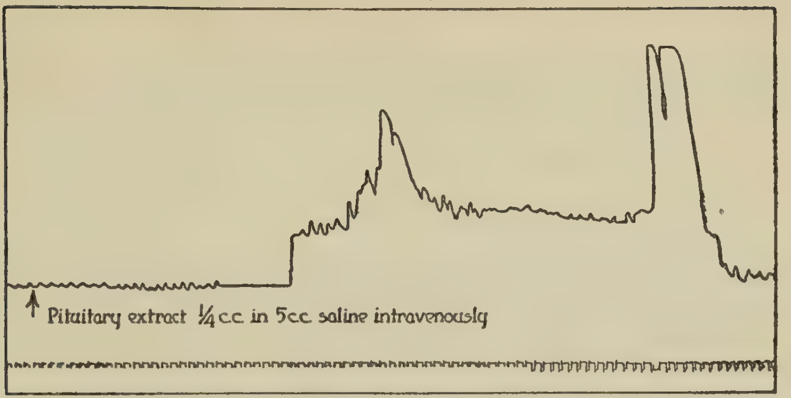

FIG. 3.—Patient: tracing thru colostomy.

tract was administered subcutaneously there occurred in each case a definite increase of intestinal activity associated with the passage of feces in from 5 to 14 minutes. In order to verify these results, 0.25 cc. was given intravenously to 2 patients with colostomies and in both of these there was definite evidence of increase of peristalsis in less than 2 minutes. (Fig. 3.)

A patient with an ileostomy was given 1 cc. of pituitary extract subcutaneously. After a period of quiescence lasting 16 minutes there was a period of increased peristalsis associated with the passage of intestinal material and gas. Subsequently he was given an intravenous injection of 0.5 cc. of the extract. In this instance the increase of peristalsis occurred in about 2 minutes.

The observations in this series of experiments corroborate the clinical impression that commercial extracts of pituitary have a definite stimulating effect upon the human colon and small intestine, especially when administered intravenously. The effect of these preparations on the intestine of the dog is inconstant, a stimulating effect being the exception rather than the rule. The intestine of the rabbit is more responsive to pituitary extract than that of either the dog or cat.

4966

Bilateral Thoracoplasty: An Experimental Study upon the Dog and the Monkey.*

THOMAS J. KINSELLA. (Introduced by Owen H. Wangensteen.)

From the Department of Surgery, University of Minnesota.

In order to determine experimentally any possible ill effects which might follow the use of bilateral upper extrapleural paravertebral thoracoplasty in the treatment of bilateral chronic apical pulmonary tuberculosis, the procedure was carried out on a series of dogs and on a monkey before attempting it on the human. All operative procedures were patterned as nearly as possible after the Wilms-Sauerbruch and Brauer technique as applied to man. The approach in dogs was made through a curved incision posterior to the scapula separating the fibres of the latissimus dorsi without cutting them and sectioning the tendon of insertion of the rhomboids at its attachment to the scapula, being careful to avoid interrupting the nerve supply of any of the structures. The technique employed in the monkey was almost identical with that used in man. A multiple stage subperiosteal costectomy of the paravertebral type was employed in all. The mortality encountered in the series was apparently in no way related to the bilateral collapse itself.

Both a symmetrical and asymmetrical type of bilateral thoracoplasty were performed upon the dogs. Dog No. 7, a small German Shepherd, illustrates the symmetrical type of collapse in which approximately 50% of the total length of the right upper 7 ribs and of the left upper 6 ribs were resected posteriorly. The asymmetrical group is represented by surviving dog No. 4, a Great Dane, in which approximately 35% of the total rib length of the right upper 9 ribs and the left upper 4 ribs was resected in the paravertebral region. Subsequent regeneration of these ribs produced a fixation of the upper chest in the collapsed position. At the present time these animals show no gross deformity, no interference with their normal activity, no respiratory embarrassment even under moderate exercise and no spinal curvature.

A single specimen of small monkey (Rhesus) was then operated upon in order to observe any spinal changes which might occur with bilateral collapse as a result of erect posture. Large segments of the right upper 6 and the left upper 4 ribs were removed, the total

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* This work was made possible by a grant from the Graduate School of the University of Minnesota.

lengths being 25% of the amount which would be removed in man in the typical Wilms-Sauerbruch operation. Here also no gross deformity, respiratory embarrassment and no spinal deformity was noticed. The animal seems to be as active as its fellows. The amount of collapse on all of these animals will be made more extensive at a later date.

From the above observations upon animals, we may judge that bilateral upper paravertebral thoracoplasty, where indicated, may be undertaken without fear of gross deformity, of respiratory embarrassment due either to the extent of the collapse itself or to the fixation of the chest wall by regeneration of the ribs, or of spinal curvature resulting from either the symmetrical or asymmetrical multiple rib resection.

New York Meeting.

New York Academy of Medicine, May 21, 1930.

4967

Studies in Gastric Analysis in Children.

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From the Departments of Biochemistry and of Pediatrics, New York Post-Graduate Medical School and Hospital.

As a preliminary to a study of gastric function in pathological conditions in children, this investigation was undertaken to establish normal figures in a control group. Studies were made on a total of 50 children, ages ranging from 3/12 to 8 6/12 years, in the terminal stages of convalescence prior to discharge from the hospital. None had been ill from any condition associated with the gastro-intestinal tract. The diets were unrestricted. The display of apparently normal appetites excluded anorexia. Gastric contents were aspirated through a small rubber tube introduced orally 1 hour after ingestion of 40 gm. of white bread and 200 cc. of water. In a few instances this meal was modified. Fifteen cases received 6 mg. of phenol red added to a meal. The acidity of gastric contents was determined by Topfer's method. The volume of the gastric contents varied from a minimum of 10 ml. to a maximum of 356 ml. For adults a range 50 to 120 ml. is accepted as average normal. Volumes in 23 cases fell within this zone, 20 were above, and 2 below. When volume was plotted against age of the children, there was no apparent relation of volume of gastric contents to age.

In vitro the addition of 40 gm. of bread without crusts to 200 ml. of 0.1 N hydrochloric acid increased the volume of the fluid to 230 ml., i. e., the increase in volume amounted to approximately 75% of the weight of the added bread. When incubated at 37°C for 72 hours, the volume of the mixture remained 230 ml.

The chymified sediments in the gastric extractions were permitted to settle to minimum volume at room temperature and the volumes of sediment were noted. In the 36 cases the sediment has a mean volume of 48.2% of the gastric contents, between extremes of 12.5 and 88.5%.

Results for acidity of gastric contents are expressed in terms of milli-equivalents of chlorine per 100 ml. We have accepted as a normal range of total acidity of gastric contents of adults between 6.0-8.0, of the free hydrochloric acid 3.0-4.0, and combined HCl 2.5-3.0. Seventeen per cent of the children gave figures for total acidity exceeding the upper normal level for adults, 23% fell within this zone, and the remainder of the figures were below the lower normal level. The figures for total acidity ranged from 2.8 to 11.6. Of the figures for free HCl, 9% were above the upper normal level for adults, 69% were below the lower normal level for adults, in 19% an achlorhydria was found, and 2% were within the normal adult zone. Histamine was not used in these cases. Nine per cent of the figures for combined HCl were within the range accepted as normal for adults, 30% of the figures exceeded the upper normal level for adults, and 54% fell below the lower normal level. In 6% of the cases the volume of gastric content was insufficient for determination of combined hydrochloric acid.

The difference between the sum of the free hydrochloric acid and combined hydrochloric acid, and the total acidity was significantly greater than this difference noted in adults. (Maximum = 1.0 milli-equivalent of Cl per 100 ml.). The acidity not due to free HCl nor combined HCl averaged 2.0 milli-equivalents of chlorine per 100 ml. with a. d. = ± 0.92 and A. D. = ± 0.18.

There was evident no consistent relationship of total acidity nor of any of its components to the age of the children between 3/12 and 8 years.

A trace of bile was found in the gastric contents in only one of 8 cases examined.

Normal standards for peptic activity (with Rose-Givens technic) of gastric contents of adults are 10-20. Of the children 38% gave peptic activity within this range, 4% exceeded the upper normal adult level and 57% were below the lower normal adult figure. Twenty-three per cent presented figures below 5, and in 4% no peptic activity was found. It is not possible to demonstrate a definite relation of peptic activity to the concentration of free hydrochloric acid.

Six milligrams of phenol red were added to the meal in 15 cases and the concentration of phenol red in gastric contents determined after one hour. Results for phenol red are reported in terms of the fraction of the dye remaining in the stomach after one hour. In all cases except one the volume of the fluid and bread in the meal was 230 ml. In one instance 150 ml. of fluid were given. The volume of gastric contents withdrawn at the end of an hour averaged 54.8% of the meal, between extremes of 38 and 97%. The volume of sediment was noted after standing at room temperature until the maximum sedimentation has been obtained. In 13 cases the average normal of sediment was 61% of the volume of gastric contents, between extremes of 36 and 71%. The average phenol red concentration of gastric contents was 23.13% of the amount ingested (a. d. = ± 5.7 A.D. = ± 1.6%).

No effort is made at this time to interpret these results, nor to review the literature on this subject covering this age group.

4968

On the Dialysability of Proteins.

ARTHUR F. COCA.

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In several of his recent studies upon atopic hypersensitiveness (Idiosynkrasie), W. Jadassohn[1, 2] has reported the fact that the excitants of the hypersitiveness (to celery, guinea pig serum and egg white) are dialysable. In the dialysate from egg white, the presence of protein could not be demonstrated with any of the chemical tests for protein. From these observations, this author draws the conclusion that the atopens in the materials mentioned are not proteins and not antigens. He considers them therefore as belonging in the group of the haptens (Landsteiner).

Jadassohn did not directly test any of the dialysates for the presence of antigen, and he seems therefore to base his conclusion that the dialysable atopens are not antigenic, upon the universally accepted assumption that the antigens are colloids and therefore not dialysable.

Jadassohn does not state in any of his reports, what kind of membrane he used in his experiments.

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[1] Jadassohn, W., *Schweizerischen Med. Woch.*, 1926, No. 27.

[2] Jadassohn, W., *u. Zaruski. Marg., Arch. f. Derm. u. Syph.*, 1926, cli, 93.

I have used both animal membrane (fish bladder), and the collodion sac, and with these I have been able easily to confirm Jadassohn's results with pseudoglobulin, albumin, and mucoid of egg white. Other materials were not used. Each sac was tested for leakage, under moderate pressure in a dark room, with hand lens.

The dialysates collected in distilled water were concentrated by fanning to about 1/500 volume and tested with 3 antisera, which were quite specific for the 3 proteins mentioned above. All 3 proteins could be identified in the concentrated dialysates. In 2 instances, the concentration of the dialysed substance was sufficient to permit the chemical demonstration of biuret.

In one experiment with ovo-mucoid, the dialysed solution (5-10%) was quantitatively compared with the concentrated dialysate, with the specific precipitation method, and the biuret test (colorimetric); with the former, the concentrated dialysate appeared about 1/8 as strong as the dialysed solution, and, with the latter, the ratio was 1-7.

The coagulable proteins show a tendency to spontaneous coagulation after they have passed through the membrane and have been concentrated by fanning.

The antigenic property of the protein in the dialysate from pure ovo-mucoid, was demonstrated by injecting the concentrated dialysate into 2 guinea pigs. Two weeks later, one of these animals died in typical shock, on the intravenous injection of 0.2 cc. of the concentrated dialysate, and the other died, typically, after the intravenous injection of 0.2 cc. of the dialysed ovo-mucoid diluted 1-10. Two normal guinea pigs received 0.4 cc. of the two solutions intravenously, without symptoms.

The proteins in the dialysate are therefore not haptens.

The question of the permeability of the collodion membrane to protein, has been previously considered by Antropoff[3]; and Sörensen[4] found coagulable egg protein "in the outer liquid," in his experiments with the collodion cap.

The experiments here reported could not be carried out with parchment sacs because none was found, of the larger size, that did not leak under pressure.

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[3] Antropoff, A. von, *Z. f. physik. Chem.*, 1911, lxxvi, 721.

[4] Sörensen, S. P. L., *Compt. Rend. Carlsberg*, 1915-17, xii, 323.

4969

Inorganic Salts in Nutrition. III. Some Effects of Replacing Inorganic Salts in a Ration Poor in Ash.*

PEARL P. SWANSON, ROBERT V. SCHULTZ AND ARTHUR H. SMITH.

From the Laboratory of Physiological Chemistry and the Department of Pediatrics, School of Medicine, Yale University.

It has previously been shown[1] that in rats 130 days old which, for the 90 days preceding the determinations, had been given an experimental ration extremely poor in inorganic salts and whose body weight had thereby been maintained at the level of 160±10 gm., there occurred striking alterations in the blood. When compared to animals of the same age which had grown at the usual rate, the stunted rats had blood with a striking increase in the number of erythrocytes, a marked decrease in relative concentration of hemoglobin and a significant diminution in the red cell volume. These changes have been shown to be independent of the concentration of the blood, the quantity of dietary protein consumed and the amount of total food eaten and have been attributed to the marked deficiency of mineral salts in the experimental ration which was otherwise adequate.

Data are now available on a few rats which, after having been given the same experimental treatment as those animals described above, were realimented with a qualitatively complete diet. This ration was so adjusted that, although the quantity of the indispensable factors—protein, salts and vitamins—ingested was the same as that consumed by a normal rat of the same weight, the energy intake was limited to prevent increase in body weight. The observed results appear in the accompanying table.

It is seen that the red cell count decreases progressively until at the end of six weeks it is somewhat below the normal level. Furthermore, at the end of the period of realimentation both the cell volume and the hemoglobin have made substantial increases toward the normal value.

The bloods of some of the experimental animals were examined for reticulocytes. In normal rats in the stock colony and in some of the control animals on adequate rations, the values for reticu-

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* The first two papers in this series are, respectively, Smith, A. H., and Swanson, P. P., *Am. J. Physiol.*, 1929, xc, 517, and Smith, A. H., and Schultz, R. V., *Am. J. Physiol.*, 1930, in press.

[1] Smith, A. H., and Swanson, P. P., *Am. J. Physiol.*, 1929, xc, 517.

TABLE I.

	Beginning	Time Interval After Beginning Realimentation in weeks.				
		2	3	4	5	6
Average red cell count experimental rats, in millions	12.0	11.2	10.1	9.6	9.4	8.8
Average red cell count control rats, in millions	9.7					
Average hemoglobin experimental rats, gm. per 100 cc. blood	14.3					16.4
Average hemoglobin control rats, gm. per 100 cc. blood	17.4					
Average cell volume experimental rats, %	45					49
Average cell volume control rats, %	54					

locytes vary from 0% to 10%. In 5 "low salt" rats before realimentation the values were 18%, 21%, 14%, 21% and 28%; in one rat after 4 weeks of realimentation the value was 32%; in 2 rats after 5 weeks, 43% and 37%, and in the same 2 animals after 6 weeks realimentation the values were 33% and 27% respectively. Four other rats showed 26%, 18%, 9% and 4% in this period. It is apparent that, along with the other adjustments to the deficiency of inorganic salts in the ration, there occurs a marked reticulocytosis which seems to regress much more slowly than do the other abnormal conditions of the blood which were studied, possibly because the abundant salts in the ration during realimentation have acted as a stimulus to the production of reticulocytes, thus maintaining the high level observed after 6 weeks realimentation.

These observations lead to the conclusion that the hemopoietic mechanism is greatly influenced, directly or indirectly by strict limitation of inorganic salts in the diet. The data herein reported indicate further that the observed change in the blood under conditions of the experiment are due specifically to the deficiency in mineral salts.

4970

Observations on the Mechanism of Chloride Retention in Pneumonia.

ISIDOR GREENWALD.

From the Department of Pharmacology and the Littauer Pneumonia Research Fund, New York University.

In 4 experiments upon 3 dogs, following the intratracheal or intrapneumonic injection of Type I pneumococcus, there was a large increase in the excretion of chloride on the first day. Thereafter, the output was much lower than in control experiments. This finding resembles those reported by von Moraczewski[1] and by Terray[2] in regard to the increased excretion of chloride during the febrile period in malaria.

It is possible that there is a similar large excretion of chloride on the first day of pneumonia in man. This would account for the low concentration of chloride in the blood and for at least part of the marked retention usually observed. Other factors, such as the accumulation of chloride in the consolidated lung tissue and the retention of water, also play a part and account for the post-critical excretion of chloride that has so frequently been observed.

If the conditions in pneumonia should resemble those observed in these experiments, as much as 8 gm., or more, of sodium chloride might be lost on the first day of the disease. It is not surprising, therefore, that analyses of tissues of patients dead from pneumonia have failed to disclose where the retained chloride was deposited.

4971

On the Mechanism of Chemotherapeutic Action. I. Formation of the Parasitotropic Agent from Arsenicals.

L. REINER AND CLIFFORD S. LEONARD.

From the Research Laboratories, Burroughs Wellcome and Co., Tuckahoe, N. Y.

In a prior paper[1] a method was described by which single phases of chemotherapeutic action could be studied separately*. These phases are possibly due to interactions between (1) agent and parasite, (2) agent and host, and (3) host and parasite.

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[1] von Moraczewski, W., *Virchow's Archiv.*, 1899, clv, 11.

[2] Terray, P., *Z. f. klin. Medizin*, 1894, xxvi, 346.

[1] Reiner and Köveskuti, *Deutsche med. Wochschr.*, 1927, liii, 1988; *Orv. Hetilap.*, 1928.

Working[1] with Bayer 205, weak solutions (0.5% and 0.05%) were allowed to act upon trypanosomes (*Tr. equiperdum*); then the parasites washed several times with a saline-broth mixture and rats infected with them. Control rats were infected with trypanosomes which had been treated similarly with saline-broth mixture only. If a small number of untreated trypanosomes were injected together with 10 times as many treated trypanosomes into one rat, and the small amount of untreated trypanosomes only into another, in all experiments both animals died at the same time and much earlier than rats which were infected with the large amount of treated trypanosomes only. This proved that Bayer 205, once bound, could not act upon other trypanosomes. Thus with Bayer 205, a direct action of the chemotherapeutic agent upon the parasites was demonstrated; only a decrease of virulence results. Similar experiments with arsenicals are described in the present paper.

It was to be expected that in this type of experiment, arsenicals would behave similarly to Bayer 205. Such was not the case. If an emulsion of trypanosomes was brought in contact with neoarsphenamine dissolved in nutrient broth in a dilution of 1/5000, or with sodium atoxylate, and allowed to stand for 20 minutes, then centrifuged and washed, the trypanosomes remained almost as virulent as the controls. Time of action and dilution of the chemotherapeutic agent were chosen far below the limits which produce a damaging effect as shown by the change in motility.

These experiments show that the arsenicals investigated either were not bound to the parasite, or, if bound were not active as chemotherapeutic agents. However, since activity is usually seen when these compounds are injected into infected animals, it seemed probable that an interaction occurs between the chemotherapeutic agent and the host, resulting possibly in the production of a new active compound. The findings of Swift and Ellis[5] and Stühmer[6] indicated that such compounds might be present in the blood of a non-infected, healthy animal, treated with neoarsphenamine. Corresponding experiments were conducted in 3 stages: (1) treatment of a normal animal with the arsenic compound; (2) treatment of the trypanosomes with the serum of an animal treated with the chemotherapeutic agent; (3) infection experiments, in the manner described above, with trypanosomes treated with the plasma or serum from rats and rabbits, both arsenic treated and untreated.

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* Even *in vitro* experiments like those of Castelli[2] and Gonder[3] do not allow any conclusions to be drawn as to the interaction between chemotherapeutic agent and host and are thus not absolutely conclusive as to the direct action either. Objection to some of the conclusions very recently arrived at by G. A. Cooper[4] may also be based on failure to separate the action of the parasite on the host from the action of the arsenical on the host.

[2] Castelli, *Z. Chemotherapie*, 1913, i, 122, 321.

[3] Gonder, *Z. Immunitäts.*, 1912, xv, 257.

[4] Cooper, G. A., paper read before the Pharmacological Society at Chicago meeting, March, 1930.

In Stage 2 no *in-vitro* parasitocidal action could be detected but in Stage 3, trypanosomes treated with neoarsphenamine-plasma or serum showed, in accordance with the experiments of Swift and Ellis[5], a distinct protection as compared to infections with the same number of trypanosomes treated similarly with normal plasma or serum. If more trypanosomes were injected (about 1,000,000) the animals infected with treated trypanosomes lived 1 or 2 days longer than the controls. If the number of trypanosomes injected did not exceed 100,000 the controls died, whereas the animals infected with treated trypanosomes usually survived.

Whereas, in the case of Bayer 205 the chemotherapeutic action is due to 2 factors, namely direct action of the chemotherapeutic agent on the parasite and protective action of the host (reticulo-endothelial system), in the case of arsenic compounds an interaction between the host and the chemotherapeutic agent must also be assumed, since, *compared with the arsenic content, a very active parasitotropic agent is present in the serum or plasma of animals treated with neoarsphenamine. This agent acts by the same mechanism as Bayer 205.* The active compound is probably a loose combination of serum protein (globulin) and neoarsphenamine, for it is also formed *in vitro* by mixing neoarsphenamine, with serum or plasma.† Atoxyl and tryparsamide are, however, as little active with serum as either substance in broth. Results, analogous in principle to those obtained with neoarsphenamine, were also found with bismuth thiosulphate.

We have said nothing as to the forces which may be concerned in binding the active chemotherapeutic agent to the parasite, nor those concerned in the decrease of virulence which results. We have not proposed a precise chemical mechanism for the reaction by which the chemotherapeutic agent is formed. Possibly the sulfhydryl mechanism is involved in one or all of these reactions.[7] Further study of various chemotherapeutic agents and heavy metal compounds is in progress.

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[5] Swift and Ellis, *J. Exp. Med.*, 1913, xviii, 435.

[6] Stühmer, *Münch. med. Wochschr.*, 1914, dccxlv, 1101, and 2338.

† It is obvious that Castelli and Gonder were investigating this combination.

4972

Mechanism of Chemotherapeutic Action. II. Rôle of Reticulo-endothelial System in Formation of a Parasitotropic Agent from Arsenicals.

L. REINER, CLIFFORD S. LEONARD AND S. S. CHAO.

From the Experimental Research Laboratories, Burroughs Wellcome and Co., Tuckahoe, N. Y.

Recently many investigators[1-10] have attempted to study the rôle of the host in chemotherapeutic action by investigating the influence of blockade of the reticulo-endothelial system. They found that different chemotherapeutic agents were less effective with blocked, or blocked and splenectomised animals than with normals. From prior experiments[11] it appears that even as active an agent against trypanosomes as Bayer 205 needs the host's normal protective mechanism for the destruction of the parasites. Thus it was to be expected that blockade would influence unfavorably the course of an infection whether or not a chemotherapeutic agent was also applied. Confirming this view, Kikuth and Regendanz's experiments indicate that treated and non-treated infections are influenced similarly by the blockade. On the other hand, it is known[12] that arsenicals, especially the less diffusible, trivalent compounds are bound and stored in liver and spleen. It was noted especially by Voegtlin,[13] Dyer and Miller that storage favors the chemotherapeutic action. Theories have also been advanced according to which the chemotherapeutic agent stimulates the reticulo-endothelial system[7] or this system inhibits the agent from exerting its action[8] or transforms it into a more active agent. There are no experimental proofs that the influence of the reticulo-endothel is more than what one could expect from its known functions in intermediary metabolism and in detoxification.

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[7] Voegtlin, Dyer and Leonard, *U. S. Public Health Rep.*, 1923, xxxviii, 1882; *J. Pharmacol. Exp. Therap.*, 1925, xxv, 297.

[1] Kritschewsky, I. L., and Meersohn, J. S., *Z. Immunitäts.*, 1926, xlvii, 407.

[2] Kritschewsky, I. L., *Z. Immunitäts.*, 1927, liii, 506; *Centr. Bakt. Parasitenk. Orig.*, 1927, civ, 214; *Z. Immunitäts.*, 1928, lix, 1.

[3] Kritschewsky, I. L., Baskin, M. M., and Lebedjeva, M. N., *Centr. Bakt. Parasitenk. Orig.*, 1930.

[4] Kolpikow, N. W., *Z. Immunitäts.*, 1926, xlviii, 182.

[5] Jungeblut, Cl. W., *Z. Hyg. Infektionskrankh.*, 1927, cvii, 357.

[6] Jungeblut, Cl. W., and McGinn, Barbara R., *J. Exp. Med.*, 1930, li, 5.

[7] Feldt and Schott, *Z. Hyg. Infektionskrankh.*, 1927, cvii, 453.

[8] Jancso, N. v., *Z. ges. exp. Med.*, 1926, lxi, 63; 1929, lxv, 98.

[9] Schlossberger, H., *Centr. Bakt. Parasitenk. Orig.*, 1929, cx, 210.

[10] Kikuth and Regendanz, *Z. Immunitäts.*, 1929, lxi, 422.

[11] Reiner and Köveskuti, J., *Deut. Med. Wochschr.*, 1927, liii, 1988.

[12] Voegtlin, C., and Thompson, J. W., *J. Pharmacol. Exp. Therap.*, 1922, xx, 85.

Since we[14] have demonstrated that arsenicals are transformed in the host into a more active parasitotropic agent, we thought to obtain some idea of the importance of the reticulo-endothel in chemotherapeutic action by investigating its influence on formation of this active agent in non-infected, arsenical-treated animals. Thus we eliminated the influence of the blockade on the infection and were permitted to study separately its possible influence upon the chemotherapeutic agent.

Our procedure was as follows: Animals (rats and rabbits) were injected intravenously with 5 cc. per kilogram of india ink, diluted 1:5 with physiological salt solution. Twenty hours later these animals and controls received equal amounts of arsenicals intravenously (sodium atoxylate was given intraperitoneally). Doses: 100 and 250 mg. per kilogram. One day later (in one experiment, 3 hours later) the animals were bled. The plasma of blocked and non-blocked animals was diluted with 3.5% sodium citrate in the same proportion (1:2 or 1:3). These plasma were tested for their activity by the combined *in vitro* and *in vivo* method, described previously,[14] using for each blood sample 2-3 rats. Virulence controls of the trypanosomes were treated similarly with normal plasma, likewise diluted in the same proportion with citrate. In cases where the effect was slight the test was later repeated with the same blood sample and always with exactly the same result, indicating a high accuracy and sensitivity of the method and that the virulence of the control trypanosomes was kept unchanged. The arsenic content of the blood-plasma, spleen and liver was estimated by the Gutzeit method. For relative estimation of neoarsphenamine, we also used the method suggested by Hiramatsu.[15] It was never found that the blocked animal's blood was less active than that of the non-blocked, which would follow if the reticulo-endothel tended to enhance chemotherapeutic activity. In 3 experiments out of 5 (twice with neoarsphenamine, once with arsphenamine) we found that the blood of blocked animals was more active than that of non-blocked. In 2 experiments the difference was small but constant on repetition. In another experiment in which 250 mg. per kilogram was injected, bleeding 3 hours later, animals lived twice as long as those infected with trypanosomes treated with non-blocked, arsenic plasma, although these also showed a marked protection. Arsenic content of plasma was in these cases higher in blocked animals than in non-blocked. The arsenic content of the liver did not differ in blocked and non-blocked animals, whereas the non-blocked spleen usually contained more arsenic than the blocked.

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[13] Voegtlin, C., Dyer, H., and Miller, *J. Pharmacol. Exp. Therap.*, 1922, xx, 129.

[14] Reiner, L., and Leonard, C. S., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 788.

[15] Hiramatsu, *Sci-i-Kwai Med. J.*, xlviii, 133; *Eng. Abst.*, 1929, Sect. 5.

We conclude that *"blockade" does not inhibit the formation* of the parasitotropic agent in the host, but rather may enhance it. Since the blockade tends to increase the arsenic content of the blood of animals treated with arsenicals, it is probable that the increased activity is due to an increased concentration of the active principle. But the possibility should not be overlooked that other changes of the blood plasma produced by the blockade[16] may have influenced the results of our experiments. That the reticulo-endothelial system was "stimulated" is not probable, for we used large doses of india ink and found changes in the distribution of arsenic which must be attributed to a fairly high degree of blockade.

4973

Relation of Temperature to Nutrition and Resistance.

I. J. KLIGLER AND A. GEIGER.

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With our studies on the effect of external environment on susceptibility and resistance of animals to infection, we are also studying the indirect effect through the influence on the nutrition. We[1] found that rats fed high fat-containing diets (20-35% of the calory requirements) in the form of vegetable oil or cocose, showed marked loss in weight and other unfavorable symptoms as well as decreased resistance to infection. These effects appeared to be more marked during the summer than during the fall season of the year. We, therefore, undertook a more systematic study of the relation of external environment to nutritive requirements, namely the relation of temperature to the metabolism of vegetable fats.

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[16] Wichels, P., *Z. ges. exp. Med.*, 1926, liii, 287.

[1] Geiger, A., Mueller, R., and Kligler, I. J., *Biochem. Z.*, in press.

The procedure consisted in observing parallel groups of rats kept at different temperatures and maintained on identical diets. The animals were about 6 weeks old at the start of the experiment and received a daily ration of 22 to 25 calories. The following is a typical experiment:

Four groups of rats, 3 males and 3 females per group, were used. Two of the groups were kept at a temperature range of 17-20°C. and the other two at 28-30°C. The diets were as follows:

The rats of cage I (animal house) and cage III (incubator) received daily:

Whole wheat bread	5.4 gm.
Milk	2.0 "
Casein	0.5 "
Olive oil	1.0 "
Total	8.9 "

The rats of cage II (animal house) and cage IV (incubator) received daily:

Whole wheat bread	5.4 gm.
Milk	2.0 "
Casein	0.5 "
Olive oil	0.25 "
Total	8.15 "

TABLE I.

Duration of a Trypanosome infection* in rats maintained on various fat diets at different temperatures.

No. of cage	No. of rats	Weight at time of infection	Temperature	Diet: olive oil gm.	Incubation period Ave. days	Illness Ave. days
I	6	76.5 gr.	17-20°C	1.00	3.3	11.6†
II	6	72.5 ”	17-20°C	0.25	5.3	15.0
III	6	56.5 ”	28-30°C.	1.00	3.16	10.2
IV	6	68.5 ”	28-30°C.	0.25	3.66	13.0

* Each rat received 32,000 *Tr. evansi* intraperitoneally.

† The median durations of infection were as follows: I = 11 days; II = 15.5 days; III = 10.2 days; IV = 13.5 days.

The results of average weight at 13 weekly intervals shows that while at 20°C. there is relatively little difference in the growth curves of the 2 sets of rats, at 28°C. the difference is quite marked.

At the end of the experiment these rats were infected with *Tr. evansi.* The results, summarized in Table I show that the duration of the infection parallels the growth curves. The animals showing a better growth curve are more resistant, while those showing a retarded growth curve manifested a lower resistance.

These experiments indicate that not only does the external temperature influence the resistance of animals directly[2] but that it may do so indirectly by its effect on metabolism. These experiments are also suggestive in connection with the whole problem of climate and nutrition—a problem which has heretofore not been a subject of experimentation.

4974

Experimental Ostitis Fibrosa Cystica in Dogs.

HENRY L. JAFFE AND AARON BODANSKY.

From the Laboratory Division, Hospital for Joint Diseases, New York City.

In previous reports the production in the guinea pig of fibrous bone lesions by injection of parathyroid extract was described, as well as its effects on the serum calcium and phosphorus.[1,2] The production in dogs of *ostitis fibrosa cystica* is possible, but more difficult. The difficulty lies in the fact that in the dog doses of parathyroid extract (Parathormone Collip) necessary to produce marked resorption of the bone and marrow injury, are liable to lead to fatal hypercalcemia before there is much fibrous repair.

We studied eleven growing puppies for periods from 10 to approximately 180 days. They were under the influence of increasing doses of parathormone. We produced, depending upon the dosage and the length of time under parathormone, all degrees of change from mild bone resorption and slight fibrous replacement of the marrow to severe bone resorption and degeneration of the marrow with hemorrhage, when the animals died from overdosage. Finally, we produced typical *ostitis fibrosa cystica* in 3 dogs that were injected for 5 to 6 months. In these we gradually increased the daily dose of parathormone. At the end of the experimental period they were receiving 20 units daily.

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[2] Kligler, I. J., and Olitzki, L., *Science*, 1929, lxx, 45; *Am. J. Hyg.*, in press.

[1] Jaffe, H. L., Bodansky, A., Blair, J. E., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 710.

[2] Bodansky, A., Blair, J. E., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 708.

The dogs with the more pronounced lesions showed: (1) resorption of the existing spongy and cortical bone, (2) invasion of the enlarged haversian spaces and of the marrow canal by fibrous tissue, (3) the presence of Howship's lacunae, containing osteoclasts, on the walls of the haversian spaces, on the inner and outer surfaces of the compacta, and on the surfaces of the spongy trabeculae, (4) new bone formation (osteoid tissue), as a substitute for the original lamellar bone, and (5) cysts and hemorrhages in the marrow cavity. Some of the bones were deformed, particularly those of the forelegs.

One of the most marked clinical features in all the dogs was the stunting of their growth, which was referable to a cessation of bone formation and growth at the epiphyseal cartilage plates. The bone changes were generalized, and the ribs offered very satisfactory material for study. In the long tubular bones, the changes were most marked in the diaphysis and metaphysis. During the study frequent blood examinations were made, particularly in regard to the serum calcium and phosphorus, but we found that loss of appetite was a very good indication of the existence of hypercalcemia and parathormone overdosage. If these existed the parathormone was omitted for short periods, allowing the dogs to recover.

The diet consisted of lean meat, which was fed daily in ample quantity. This was supplemented with 1 cc. of cod-liver oil, tomato juice, and during various phases of the experiment calcium was given in the form of calcium lactate by stomach tube or calcium lactate and bone meal were added to the meat. This is an adequate diet.

It takes several months to produce fibrous changes in the bones of the dogs. These fibrous changes may be produced even when the calcium intake is high. In one dog, receiving 650 mg. of calcium daily in the form of calcium lactate administered by stomach tube, fibrous replacement of the bone was observed even on 8 units of parathormone daily. The production of *ostitis fibrosa cystica* was enhanced in the dogs by depleting their calcium stores through the diminution of the calcium intake, which permits giving larger doses of parathormone without danger of hypercalcemia.[3] That the changes observed could not be attributed to calcium deficiency alone is evident from examination of bones of control dogs kept for months on a low calcium diet. These animals developed osteoporosis, which is histologically quite different from *ostitis fibrosa cystica.*

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[3] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 797.

4975

Experimental Chronic Hyperparathyroidism in Dogs Without Hypercalcemia.

AARON BODANSKY AND HENRY L. JAFFE.

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The rate at which the elimination of tissue and bone calcium proceeds in the dog under the influence of parathormone depends, among other factors, upon the dosage. However, the dose of parathormone that may be administered to the dog is limited by the danger of fatal hypercalcemia.

Serum calcium is obviously one term in an equilibrium. Some of the other terms affecting it, under given physico-chemical conditions of the blood, are: tissue calcium and other salts, bone salts, their availability, and the rate of calcium excretion, particularly by the kidneys. The rapid rate of excretion of calcium, as well as dietary factors, are probably largely responsible for the phenomena observed by us in the guinea pigs after parathormone. We have shown[1, 2] that this animal tolerates large doses of parathormone, although it reacts to the extract both by an elevation of serum calcium and by formation of bone lesions. In our belief, this "tolerance" is the reason for the ease with which the typical changes of *ostitis fibrosa* were produced in guinea pigs. We have succeeded, after having depleted bone and tissue calcium in dogs, in administering to them relatively large doses of parathormone without producing hypercalcemia. By thus increasing the "tolerance" of the dog to parathormone it was possible to produce *ostitis fibrosa cystica* in this animal.[3]

This report is based on a study of 11 growing dogs in which experimental chronic hyperparathyroidism was produced by daily in-

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[1] Bodansky, A., Blair, J. E., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 708.

[2] Jaffe, H. L., Bodansky, A., and Blair, J. E., *Ibid.*, 710.

[3] Jaffe, H. L., and Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 795.

jections of parathormone. The diet of the dogs consisted of an adequate quantity of fresh raw meat with additions of cod-liver oil, bone meal, calcium lactate and tomato juice. Parathormone was injected at first in a daily dose of 2 units per kilo, which was raised gradually in some dogs to as high as 5 units toward the end of the experimental period of 5 to 6 months.

The first injections of parathormone raised the serum calcium to about 15 or 16 mg. per 100 cc. It was sometimes necessary to interrupt the treatment because of vomiting, loss of appetite and other symptoms of overdosage. As the treatment was continued, the serum calcium fell to normal values or lower, and larger doses could be given with only a temporary elevation of serum calcium. This condition was produced most rapidly in one dog on a low calcium intake. In that dog, indeed, serum calcium dropped to as low as 7.8 mg. per 100 cc., and seemed to be associated with a tissue calcium lowered sufficiently to account for the tremors and tetany observed in that dog. In other dogs the serum calcium was maintained at a "normal" calcium level when increasing doses of parathormone were administered, with only temporary increases after an increase of dose. These normal values, as well as the hypocalcemia observed in one instance, cannot be explained by "immunity" acquired as a result of previous parathormone treatment. When the calcium reserves were allowed to replenish after discontinuance of parathormone for a short period, parathormone injection had its usual effect of raising the serum calcium. An increased rate of excretion of calcium in the urine produced in the course of parathormone treatment is a possibility which we have not yet tested, but which we suggest tentatively.

We have found in the literature of parathormone therapy instances in which we suspect the presence of hyperparathyroidism, although hypercalcemia was not observed. In view of the reliance that is frequently placed upon hypercalcemia as a sole criterion of hyperparathyroidism we should be justified in designating hyperparathyroidism without hypercalcemia as *crypthyperparathyroidism.* This condition is to be recognized by a negative calcium balance and other evidences of hyperparathyroidism in the absence of hypercalcemia.

4976

Studies of the Relation of the Pituitary to Watermetabolism.

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Recent studies of Hoff and Wermer[1] reveal the presence of increased quantities of pituitary hormone in the cerebrospinal fluid after administration of diuretics. They explain their observation of increased liberation of antidiuretic substances from the posterior pituitary lobe, as an attempt on the part of the body to prevent excessive loss of water. In other words, it is a compensatory process.

The following studies were made to reveal whether there are demonstrable changes in the hypophysis after the administration of diuretics and whether such changes precede or follow the loss of water.

The experiments were made on guinea pigs of the same breed and weighing from about 250 to 600 gm. Only male animals were used. We used novasurol in a dilution of 1 to 25 of the commercial preparation. One cc. was given subcutaneously to each animal. We kept the guinea pigs without food after the injection. They were killed at intervals of 20 minutes, 40 minutes and 1, 2, 3, and 6 hours. Their hypophysis was removed, fixed in Zenker's solution and studied histologically.

We determined the loss of water by weighing the animals before and after administration of the diuretics. Weighing was chosen as our method in order to account also for loss of water by ways other than diuresis.

Normal guinea pigs kept without food lose 3.5% of their body weight as an average within 6 hours. In contradistinction to this, the animals which were given novasurol lost 5.2% of their weight within the experimental period of 6 hours. These figures tallied well with those obtained on a series of white mice. The average loss of weight in normal mice kept without food for 6 hours was 3.6% whereas mice after the administration of novasurol lost 4.8% weight in the same time.

In removing the hypophysis of the guinea pigs we noticed that 40 minutes after novasurol was given, there was already a distinct hyperemia of the anterior lobe as compared with the controls. This was corroborated by the histological findings which showed wide sinuses filled with blood in between the trabecles of the pituitary cells.

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[1] Hoff, H., and Wermer, *Arch. f. exp. Path. and Pharm.*, 1928, cxxxiii, 84-87.

We could not determine any changes in the structure of the posterior lobe but in the anterior lobe there were changes which seemed to differ according to the time which had elapsed between the injection of novasurol and the killing of the animal. These changes involved alteration of the size of the chromophil cells, the quantity of their granules, the relation of chromophil cells to chief cells, the relation of eosinophils and basophils and finally the appearance of vacuoles in the cell bodies. These changes were already noticeable to a certain extent after 20 minutes, they were well marked after 40 minutes and could be observed throughout the experimental period. The most important feature seems to us the decrease in the number of eosinophil cells with a marked increase of basophils. This change persisted after 6 hours at which time most of the other alterations were subsiding. There was not only a decrease in the number of eosinophils but also in the quantity of eosinophil substance. Many cells showed only a crescent shaped eccentric eosinophil seam, while the rest of the cell body did not contain any chromophil substance. In other cells there appeared a perinuclear area of an amphophil or distinctly basophil color reaction. The late stage of these changes is particularly characterized by the appearance of numerous vacuoles in both types of chromophil cells. Moreover there were occasional large cells present with chromophobe cytoplasm which may also contain vacuoles.

These changes are quite comparable to those seen in other glandular organs whose function has been stimulated. They apparently express increased activity, such as the discharge of glandular cell products. Therefore it seems that the injection of novasurol acts as a stimulant upon the cells of the anterior pituitary. This effect is noticeable at a time preceding the onset of the diuresis, at least if we take the accepted figures which show that diuresis follows 45 minutes after the injection of the diuretic and reaches its maximum in from 1½ to 2 hours. (Hoff and Wermer). According to our experiments, however, there was a noticeable loss of weight in 20 minutes. This was possibly due to defecation and urination following the psychic effects of handling the animals.

We have repeated the same experiments on another series of animals to which theobromin was given instead of novasurol. The results were quite similar, showing that such changes of the pituitary can be obtained by diuretic substances of very different chemical character.

Comparing our observations with those of Hoff and Wermer we are impressed by 2 apparently paradoxical facts. First, that diuresis is accompanied by changes in the anterior lobe, whereas Hoff and Wermer determined the appearance of antidiuretic substances from what seemed to be of posterior lobe origin. Second, that these changes precede or at least coincide with the onset of diuresis. This would contradict the explanation of Hoff and Wermer according to whom the pituitary discharges its hormone in order to check a further loss of water.

The rôle of pituitary activity in the production of diuresis permits of two different explanations. It is known since the first contributions of Magnus and Schaefer[2] that pituitary extracts occasionally produce diuresis instead of oliguria. Frank,[3] Oehme,[4] Fromherz,[5] Pentimalli[6] and others maintain that the effect of these extracts is biphasic, i. e. intravenous administrations of large amounts produce diuresis, while slow or subcutaneous infusion of small amounts yields oliguria. This reminds us of the biphasic reactions obtainable with adrenalin. Yet Cow[7] has demonstrated the appearance of a diuretic substance in the cerebrospinal fluid of animals, the pituitary of which had been stimulated by injection of duodenal extracts. Moreover, the observations of von Hann[8] from Goldzieher's laboratory have shown that in cases of diabetes insipidus due to lesions of the posterior lobe, polyuria ceases terminally if the lesion gradually encroaches upon and destroys the anterior lobe. This would suggest the production of a diuretic substance by the cells of the anterior lobe antagonistic in its effect to the product of the posterior lobe. The experiments presented here are in favor of this conception, yet further evidence seems necessary before the diuretic function of the anterior lobe can be recognized.

Our observations gain additional interest in view of the autopsy findings of Kraus,[9] Berblinger[10] and others who observed a considerable increase in the number of basophil cells in the pituitary in cases of hypertension and particularly of chronic renal disease.

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[2] Magnus and Schaefer, as quoted by Hoff and Wermer.

[3] Frank, E., *Klin. Wochensch.*, 1924, iii, 847, 895.

[4] Oehme, C. and M., *Deutsch. Arch. Klin. Med.*, 1918, cxxvii, 261.

[5] Fromherz, K., *Arch. f. exp. Path. and Pharm.*, 1923, c, 1.

[6] Pentimalli, F., *Sperimentale*, 1921, lxxv, 145.

[7] Cow, D., *J. Phys.*, 1914, xlix, 367, 441.

[8] von Hann, F., *Frankf. Z. f. Path.*, 1918, xxi, 337.

[9] Kraus, E. J., *Virch. Arch.*, 1923, ccxlvii, 421.

[10] Berblinger, W., *Virch. Arch.*, 1929, cclxxv, 230.

4977

Observations on Psittacosis in Mammals.

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From the Hospital of The Rockefeller Institute for Medical Research.

The observations of Bedson,[1] Krumwiede,*[2] Armstrong,[3] and their coworkers indicate that the etiological agent of psittacosis is a filter-passer and in all probability not an ordinary bacterium. Moreover, it has been shown that, in addition to parrots, certain small birds, e.g., parrakeets and love-birds, are susceptible to the disease. Furthermore, Krumwiede, McGrath, and Oldenbusch[2] have been able to produce the disease in mice and to pass it in series by means of intraperitoneal injections of emulsions made from a mixture of the liver and spleen of infected animals. In view of the fact that man is susceptible to psittacosis and that certain birds, with which most of the experimental work in the past has been done, have the disease spontaneously and may be carriers of the virus, it seemed desirable to determine if mammals other than the mouse are suitable for experimental purposes in this field of investigation. The object of the present communication is to report that rabbits and guinea pigs are experimentally susceptible to psittacosis† provided they are inoculated and handled in a certain manner.

We have been able to confirm Krumwiede's observations concerning the susceptibility of mice to psittacosis. For many reasons, however, that need not be discussed here, the mouse proved to be an unsatisfactory experimental host. Therefore, we decided to investigate the susceptibility of other mammals to psittacosis. Rabbits and guinea pigs were inoculated intradermally, subcutaneously, and intraperitoneally with infectious emulsions of liver and spleen obtained from parrots. The results were of uncertain significance, and it soon became obvious that, if these animals were to be used, another procedure of handling them would be necessary. In view of the success met with in the investigations of many viruses when the experimental animals are inoculated intracerebrally, 0.25 cc. of an emulsion of liver and spleen from a parrot dead of psittacosis was injected into the brain of a rabbit.. The animal became sick and at the proper time was sacrificed. From its brain an emulsion was made with which other animals were inoculated intracerebrally. In this manner the disease has been passed serially through 12 rabbits. With brain emulsion from the fifth rabbit in the series 4 mice (intraperitoneally) and a parrot (intramuscularly) were inoculated, and all of them developed psittacosis. A guinea pig inoculated intracerebrally with a brain emulsion from the sixth rabbit in the series became sick and was sacrificed. An emulsion was made from the brain, and other pigs were inoculated intracerebrally. In this way the virus has been passed serially through 6 lots of guinea pigs. A parrot and 4 mice inoculated intramuscularly and intraperitoneally respectively with a brain emulsion from a pig in the fifth group of the series developed psittacosis.

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* The material with which we are working was obtained from Dr. Krumwiede, who supplied us with a parrot strain and a human strain of the virus.

[1] Bedson, S. P., Western, G. T., and Simpson, S. L., *Lancet*, 1930, i, 235 and 345.

[2] Krumwiede, C., McGrath, M., and Oldenbusch, C., *Science*, 1930, lxxi, 262.

[3] Armstrong, C., McCoy, G. W., and Branham, S. E., *Public Health Rep.*, 1930, xlv, 725.

† That we are working with the virus of psittacosis is indicated by the fact that one of the investigators (G. P. B.), having been exposed in no other way than to our experimental animals, developed a typical attack of psittacosis 5 weeks after the inception of the work.

The intracerebral inoculation of psittacosis virus in rabbits and guinea pigs at times induces signs of involvement of the brain, meningitis or encephalitis. As a rule, however, the chief sign of the infection in these animals is an extremely high temperature, 104-108.5°F., occurring 2-7 days after inoculation. Such an elevation of temperature is not caused by brain emulsions from normal animals. Furthermore the virus that we have been passing from animal to animal is neither Virus III nor the causal agent of spontaneous encephalitis of rabbits, inasmuch as it is infectious for guinea pigs, mice and parrots. Moreover, ordinary aerobic and anaerobic bacteria are not present in the brain emulsions shown by animal inoculation to be infectious.

The pathological changes in the brain consist mainly of a reaction in the meninges and around the cortical blood vessels. This reaction is characterized by collections of fluid and mononuclear cells. There is probably also some degeneration of nerve cells. The pathological pictures observed in the lungs, liver, spleen, heart, intestines, and kidneys are similar to those reported as occurring in human cases that have died of psittacosis.

The results of the work indicate that we have been carrying a virus in rabbits and guinea pigs and that this virus is capable of producing psittacosis in parrots or a disease so similar to psittacosis that at present no difference can be detected.

4978

Acid Agglutination Zones of Typical and Variant Pneumococci.

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The present basis for differentiation between typical and variant forms of pneumococci involves a subjective opinion of colony morphology. It became desirable, in the course of our studies, to develop a method for this differentiation depending entirely on some objective physical measurement. Such a method has been found to be available by utilization of the phenomena of agglutination of organisms in solutions of varying acidity. This method has been of service in recognition of variants of other bacterial species.[1, 2] Gillespie[3] has studied the acid agglutination zones of several types of typical pneumococci and found them to be similar.

The organisms to be tested were grown in pneumococcus broth, the period of incubation being kept as short as consistent with adequate growth, usually about 8 hours. The organisms were packed by centrifuging and washed 3 times with saline. After the final centrifugation, they were restored to original volume with saline. 0.5 cc. quantities of this suspension were added to a similar volume of standard buffer solution of the desired acidity, incubated for 3 hours at 56°, and then recorded. The buffer system used was that of Northrup and deKruif.[4] Results obtained with various strains are shown in the accompanying table.

These results indicate that the zones of acid agglutination for variant strains of pneumococci, either occurring spontaneously or derived through laboratory manipulation, are widely separated from those of typical strains. The strains possessing the wide zone of complete agglutination possess also the characteristics accompanying the "rough" colony form.

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[1] Webster, L. T., and Burn, C. G., *J. Exp. Med.*, 1926, xliv, 343.

[2] Hughes, T. P., *J. Exp. Med.*, 1930, li, 225.

[3] Gillespie, L. J., *J. Exp. Med.*, 1914, xix, 28.

[4] Northrup, J. H., and de Kruif, P. H., *J. Gen. Physiol.*, 1922, iv, 639.

TABLE I.

Acid Agglutination Zones of Pneumococci.

Strain	pH										Description
	2.1	2.3	2.7	3.0	3.6	4.1	4.7	5.1	5.6	6.3	
I	0	0	0	0	0	0	0	0	A	A	Type I, virulent, smooth colony
II	0	0	0	0	0	0	0	0	A	A	Type II, ,, ,, ,,
III	0	0	1	1	1	0	0	0	0	A	Type III, ,, ,, ,,
736	1	0	0	0	0	0	0	0	0	A	Group IV, ,, ,, ,,
750	0	0	0	0	0	0	0	0	0	A	Group IV, ,, ,, ,,
735	0	0	0	0	0	0	0	0	0	A	Group IV, ,, ,, ,,
R 1	0	0	0	0	4	4	4	0	0	0	Rough, avirulent, no serum specificity
R 2	0	0	0	0	4	4	4	0	0	0	Rough, avirulent, no serum specificity
I R	0	0	0	0	4	4	4	0	0	0	Rough, avirulent, no serum specificity, derived from Type I
II R	0	0	0	0	4	4	4	0	0	0	Rough, avirulent, no serum specificity, derived from Type II
III R	0	0	0	2	4	4	4	4	A	A	Rough, avirulent, no serum specificity, derived from Type III
78	0	0	0	0	0	0	0	0	A	A	Type I, avirulent, smooth colony
78 R	0	0	0	0	4	4	4	4	0	0	Rough, avirulent, no serum specificity. Strain 78 after 6 passages in Type I antiserum
734	0	2	4	4	4	4	4	2	2	2	Rough, avirulent, no serum specificity. Spontaneously occurring

0 = No agglutination or autolysis.
2 = Partial agglutination.
A = Marked autolysis.
1 = Trace of agglutination.
4 = Complete agglutination.

4979

Effect of Continued Administration of Iodide on the Growth of the Albino Rat.*

LAFAYETTE B. MENDEL AND HUBERT BRADFORD VICKERY.
(With the Collaboration of Helen Cannon Cronin.)

From the Biochemical Laboratory of the Connecticut Agricultural Experiment Station and the Laboratory of Physiological Chemistry, Yale University, New Haven.

Early in the course of the attempts in this laboratory to prepare a mixture of inorganic salts that might be adequate as a source of the mineral nutrients in feeding experiments with purified food materials, the inclusion of small amounts of iodine was planned.[1] This is reflected in the widely used Osborne-Mendel (IV) salt mixture[2] that contains 0.0025% of iodine. The quantity of iodide thus included was somewhat arbitrarily chosen, because a suitable guide as to the precise needs of the animals (rats) under investigation was not available.

Hanzlik, Talbot and Gibson[3] published a report on the comparative effects of prolonged administration of iodide and of other salts on the growth and body weight of rats. They concluded that "the continued administration of iodide in small daily doses in food over long periods (covering from about one-seventh to seven-twelfths of the span of life) to rats caused moderate though variable increases in weight and growth of the body in the majority of animals on complete dietary." The effects of other salts—sulphocyanate, bromide, arsenite, etc.—"were found to be in marked contrast to those of the iodide, thus emphasizing the distinctiveness of iodide, which was found to be beneficial and at least not harmful."

Careful studies on growing chicks at the Illinois Agricultural Experiment Station[4] have failed to show "any apparent difference either in the health or rate of growth of the birds" when a good mixed grain ration was supplemented daily in the case of some of

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* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[1] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1913, xv, 311.

[2] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxiv, 131.

[3] Hanzlik, P. J., Talbot, E. P., and Gibson, E. E., *Arch. Int. Med.*, 1928, xlii, 579.

[4] A Year's Progress in Solving Farm Problems of Illinois, 1927-28. Univ. of Ill. Agric. Expt. Station, 1928, p. 173.

the animals with 0.5 to 1 mg. of potassium iodide for each 100 gm. of body weight.

The subject is important not only pharmacologically but also in planning adequate rations for use under the restricted conditions of laboratory feeding tests or for nutrition of domestic animals in confined quarters. The necessity, or wisdom, of supplementing foods with iodine in goitrous or semi-goitrous regions is well known. There are claims, too, that iodide may improve the assimilation of nitrogen, phosphorus and possibly calcium in growing animals.[5] Chidester, Eaton and Thompson[6] have reported that "while thyroid extract and iodine will produce rapid metabolism resulting in small animals, minute doses of the iodides or desiccated thyroid prove beneficial to rats on a *normal* diet and induce bone growth and increased weight."

The experiments of Hanzlik, Talbot and Gibson attracted our attention because the iodide supplements that they fed were added to rations that already contained 4% of the Osborne-Mendel salt mixture with its 0.0032% of potassium iodide. On the assumption of an average daily food intake of 7 gm. per rat for the *unsupplemented* conventional food mixtures in which these proportions of the Osborne-Mendel salt mixture are included, the daily iodine intake will be about 0.007 mg. To this Hanzlik, Talbot and Gibson added sufficient sodium iodide to correspond to "about 1 mg. daily per rat or about 3.3 mg. daily per kg. throughout the major part of the life of the rats. This dosage of iodide would correspond to about 0.23 gm. (3½ grains) daily for an adult of 70 kg. and might represent the daily amount used during the course of medication with iodide for the prevention of simple goiter, and that used in the treatment for toxic goiter and for chronic inflammations such as bronchitis, asthma and arthritis, but would be several times greater than the daily dose of iodide used in the form of iodized table salt."

The somewhat augmented growth reported as the result of this greater iodine intake led us to investigate the possible desirability of improving the Osborne-Mendel salt mixture by increasing its content of iodide. Male albino rats in separate cages were fed from the time when they weighed about 60 gm. on a food mixture consisting of:

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[5] Kelly, F. C., *Biochem. J.*, 1925, xix, 559.

[6] Chidester, F. E., Eaton, A. G., and Thompson, G. P., *Science*, 1928, lxviii, 432.

	%
Casein	35
Cornstarch	37
Lard	15
Butter fat	9
Osborne-Mendel salt mixture	4

plus 10 drops of cod liver oil and 0.2 or 0.3 gm. dried yeast (in tablet form) daily.

The daily dosage of yeast was increased from 0.2 gm. to 0.3 gm. after each rat had been on the diet 49 days (Series A) and 56 days (Series B) respectively. Some of the animals served as "controls" without supplementary iodide; others received a daily addition of 1.107 mg. of potassium iodide dropped, in solution, upon the yeast tablets. These were readily consumed so that a fixed dosage of iodide became possible. Series A included 3 "control" rats and 6 animals that received potassium iodide. All were kept on the diet more than 400 days, a considerable part of the animals' span of life. Series B included 6 rats in each of the 2 groups for comparison over a period of 200 days. The variations between the records of individual animals are not inconsiderable; but the graphs that represent the averaged rates of gain for the respective groups are, we think, too nearly alike to permit claims of superiority for either type of diet. Unfortunately the number of animals observed in each series is too small to offset the effects of variability due to unsatisfactory growth of a few individuals.

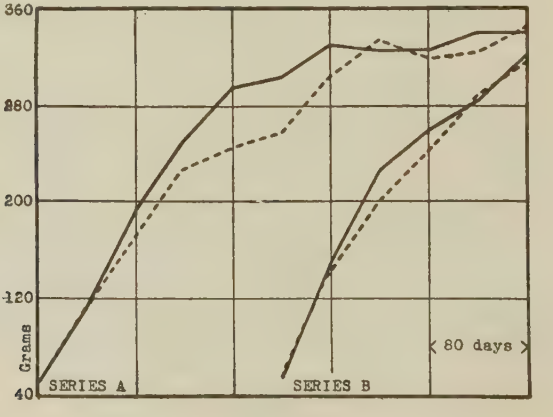

FIG. 1.

Graphs of the averaged rates of gain in body weight of comparable groups of rats. Those that received supplementary iodide are represented by the solid line; the control group by the broken line.

Whether differences between the iodine content of the water and of the "accessory" foods (yeast, alfalfa, cod liver oil) used in the two laboratories might explain the small differences in outcome is not apparent. One feature already emphasized by Hanzlik, Talbot and Gibson is particularly noteworthy, namely, the apparent harmlessness of very prolonged administration of iodide.

Mr. G. B. Moment of the Osborn Zoology Laboratory, Yale University, has kindly made histological examinations of the thyroids of all of the rats observed. He reported the absence of detectable differences in structure; on the other hand rats raised in our laboratory on diets without any additions of iodine other than that present in the natural foods and drinking water used showed somewhat defective thyroids.

4980

Vital Staining With Methyl Red.

ROBERT CHAMBERS.

From Washington Square College, New York University

A generally accepted hypothesis advanced to explain the permeability of protoplasm to certain substances is that these substances penetrate because of the solubility of their associated molecules in the plasma-membrane of the living cell.

This hypothesis would account satisfactorily for the vital staining of the basic dye, neutral red, which is a chloride or iodide of a colored organic base. At the pH of the normal medium of living cells, viz., a pH greater than 7.0, this dye is presumably in its least dissociated state and it readily penetrates and accumulates within the cells. When the stained cells, e.g., marine ova, are placed in a medium having a pH of about 6.0 which is lower than that of the protoplasm[1, 2] the color quickly washes out. Moreover, vital staining with this dye does not occur when the outside medium is more acid than the protoplasm. Apparently, therefore, the dye passes readily through the plasma-membrane from a medium of a higher to that of a lower alkalinity.

An interesting case which is explicable on the same hypothesis of

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[1] Needham, J., and D. M., *Proc. Roy. Soc., London*, 1926, xcix, 173.

[2] Chambers, R., and Pollack, H., *J. Gen. Physiol.*, 1927, x, 739.

permeability is the vital staining with methyl red. This dye which has not yet been recorded as a vital stain, except in the special instances which are noted below, is an amphoteric electrolyte possessing both a carboxyl and an amino group (carboxylic benzene azo dimethyl aniline).

Because of this it exists in a dissociated state both on the alkaline and acid side of its neutral pH range which lies between pH 5.0 and 5.5. In its alkaline range there is a low dissociation of the basic group while the acid group is highly dissociated. The dissociated state of the acid group would account for the fact that methyl red is not a vital stain at the pH of fluids which surround lving cells under normal conditions, their pH being well on the alkaline side of 6.0.

The following experiments show that when the environment of living cells is brought to a pH of about 5.5 methyl red acts as a vital stain.

Samples of sea-water the normal pH of which is about 8.4 were brought to a pH of 5.0 and 6.0 by the addition of sodium acid phosphate. Several drops of Clark and Lub's standard 0.4% aqueous solution of methyl red were added to the samples and also to normal sea-water. Mature starfish eggs were then immersed in the several samples. After several minutes to half an hour the eggs were removed and washed with sea-water at the same pH. The eggs from the sea-water having a pH of 8.4 and of 6.0 were unstained, while those in sea-water at pH 5.0 were brilliantly and diffusely colored yellow and contained red granules. The colored eggs were then transferred to sea-water having a pH above 6.0 whereupon the color quickly passed out of the eggs.

Similar experiments with the fresh water *Amoeba dubia* gave analogous results, viz., they were vitally stained with methyl red only when the pH of the medium was definitely below 6.0.

The above results may be explained on the basis of penetrability of associated molecules since methyl red is largely associated in an aqueous medium in the neighborhood of pH 5.0. At a more alkaline pH the acid group dissociates and the dye behaves as a dissociated acid salt.

Schaede[3] describes the staining yellow of the protoplasm of onion-skin cells immersed in a carmine-red solution of acidified methyl red, with various organic and inorganic acids. He also found that no protoplasmic staining occurs when the medium is alkaline.

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[3] Schaede, R., *Ber. d. deutsch. bot. Ges.*, 1924, xlii, 219.

In this respect, therefore, the permeability of plant protoplasm appears to be similar to that of the material described in this paper.

Since methyl red is amphoteric and, by virtue of its basic group, should become dissociated also in the pH ranges below 5.0 attempts were made to ascertain if, in spite of this, vital staining would still occur. This was found not to be feasible. At a pH of 4.0 and below both the marine ova and the amebae become moribund. In addition the methyl red becomes increasingly insoluble.

4981

Anaphylactic Reactions Produced by Azodyes in Animals Sensitized With Azoproteins.

K. LANDSTEINER, PH. LEVINE AND J. VAN DER SCHEER.

From the Laboratories of the Rockefeller Institute for Medical Research.

In the course of investigations on inhibition effects[1] in animals sensitized with azoproteins, anaphylactic reactions were observed following the injection of azodyes containing the same specific group as the antigen. These reactions did not occur regularly, but in a considerable number of animals; in some batches of animals the results were not well pronounced.

In such an experiment guinea pigs were sensitized with an azoprotein prepared from dextro-paraaminotartranilic acid and horse serum[2] and were found to be sensitive to 0.35-0.7 mg. of an antigen made from dextro-paraaminotartranilic acid and chicken serum. To such animals were given intravenous injections of an azodye made by coupling diazotized dextro-paraaminotartranilic acid and resorcinol. Two guinea pigs injected with 5 mgs. each did not show any symptoms; of 8 which received doses from 0.5 to 2.5 mgs. all but one showed reactions which consisted, in 5 animals, in a temperature drop from 1.3° to 2.4°C. and slight anaphylactic symptoms or general weakness and paresis of the hind legs. The 3 remaining animals showed typical severe anaphylactic shock and died, one after night, one after an hour and the third after 4 minutes. Eight other animals of this batch were injected in the same way with an azodye prepared from levo instead of dextro-paraaminotartranilic acid. These showed no anaphylactic symptoms and there was only a slight temperature drop at most of 0.6°, or an increase of temperature.

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[1] Landsteiner, K., *J. Exp. Med.*, 1924, xxxix, 631.

[2] Landsteiner K., and van der Scheer, J., *J. Exp. Med.*, 1929, l, 407.

In another series of sensitized animals 2 out of 3 injected with 2.5 mg. of the homologous azodye had a significant decrease of temperature; two injected with 0.5 mg. showed no symptoms, while of 5 which received 1 mg. one became very sick (temperature drop of 3.1°C) and 3 had typical anaphylactic symptoms and died after 4, 5 and 52 minutes respectively.

In a control experiment in which guinea pigs were sensitized with an azoprotein made from levo-paraaminotartranilic acid, 8 out of 11 had a drop in temperature of 1° to 3.2°C. when injected with 1-5 mg. of levo-dye and some presented more or less distinct symptoms (weakness and paresis); whereas in 9 animals injected with the dextro dye no symptoms were observed and the temperature drop did not exceed 0.8°C.

Further investigations are being carried out in order to study the conditions which control the outcome of these experiments.

4982

Antigens Containing Peptides of Known Structure and Antigenic Properties of Azoalbumoses.

K. LANDSTEINER AND J. VAN DER SCHEER.

From the Laboratories of the Rockefeller Institute for Medical Research.

The studies on antigens synthetized from proteins and compounds of known chemical constitution[1] have so far been concerned mostly with substances of relatively simple constitution. With a view to studying the specificity of substances with longer chains and whose composition, at the same time, is related to that of natural antigens, namely proteins, azoproteins were prepared by diazotizing and coupling to proteins the following compounds: paraaminobenzoyl-glycyl-glycine, paraaminobenzoyl-glycyl-dl leucine, paraaminobenzoyl-dl leucyl-glycine, and inactive paraaminobenzoyl-leucyl-leucine (A).

Immune sera for these azoproteins were made and tested with the methods described in previous papers. While these immune sera proved to precipitate specifically the homologous antigen, group reactions were observed dependent mainly on the amino acid carrying the free carboxyl group.

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[1] Landsteiner, K., and Lampl, H., *Biochem. Z.*, 1918, lxxxvi, 343.

In another series of experiments rabbits were immunized with hetero-albumose or protoalbumose (from Witte peptone) which had been coupled with diazotized aniline. Immune sera obtained with azoheteroalbumose gave a precipitation with solutions of heteroalbumose itself which was due to the presence of an acid precipitable substance, most likely metaprotein. In addition however, the immune sera for azoprotoalbumose as well as those for azoheteroalbumose gave a distinct precipitin reaction with both these azo-antigens up to a dilution 1:10,000 of a 5% solution indicating the production of antibodies as a response to the injection of the azoalbumoses. The sera did not react upon solutions of azodeuteroalbumose (from Witte peptone). The protoalbumose preparation used contained also heteroalbumose.

4983

Further Observations on Hemagglutination by Tumor Extracts.

K. LANDSTEINER.

From the Laboratories of The Rockefeller Institute for Medical Research, New York.

The agglutinating substance found in tumor extracts[1] was not demonstrable in any appreciable amount in various organs of mice and rats, viz., liver, kidney, brain, spleen, heart. A distinctly positive reaction was obtained, especially in cysteine solutions, with muscle of rat, calf, and pig, and with the uteri of mice. Tumor and embryonic tissues were tested after drying. The dried material retains its activity for at least several weeks. The agglutinating substance of the tumor proved to be active after filtration through Berkefeld filters of a neutral, cysteine-containing solution. The active substance is absorbed by rabbit blood.

When extracts in 0.9% saline solution were heated at 55° for 20 minutes the activity was lost. This is evidently due to oxidation since in a saline solution containing 1% cysteine the agglutinating property persists and this is also the case when such solutions are brought to a boil or kept in boiling water, e. g., for 15 minutes. The substance was found to be still active when a water extract was heated to boiling, although the results were irregular. The activity of saline extracts which had been inactivated by boiling for a short time could be reactivated on the addition of a neutralized solution of cysteine to give a final concentration of 0.5%, and the same restitution effect was observed with extracts which had become inactive upon standing in the room for several hours.

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[1] Landsteiner, K., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 134.

The properties of the active substance suggested attempts to concentrate it. This could be done in the following manner: 6 gm. of rat sarcoma were ground with the addition of 5 cc. of water, and the suspension was poured into 25 cc. of boiling water, slightly acidulated with 3 drops of 5% acetic acid. After being heated to boiling, the suspension was cooled and coagulated protein removed by spinning. The supernatant fluid was neutralized and evaporated by vacuum distillation. The apparatus used for distillation was filled with pure hydrogen which was passed through 2 washbottles with alkaline solution of pyrogallol and a third bottle containing water. After evaporation to a small volume the water was completely removed in a well evacuated desiccator over P_2O_5. The dry material was then taken up in 5 cc. saline solution containing 0.2% cysteine. This solution had a stronger activity than the original tumor extract and agglutinated rabbit blood instantaneously. Moreover, in contrast to the original extract it agglutinated also sheep, horse, and guinea pig blood.

4984

Effect of Adrenalin on Blood Fat.

H. E. HIMWICH AND M. L. PETERMANN.

From the Department of Physiology, Yale University, New Haven.

The results of the first series of a group of studies on fat metabolism have been presented in preliminary form.[1] The present communication contains the results of the 4 pilot experiments of a second, projected series of these studies. Samples of arterial blood were drawn from amytalized dogs, and the fat contents of the blood plasma were determined by the method of Stewart and White.[2]

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[1] Himwich, H. E., Friedman, H., Berry, E., and Chambers, W. H., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 193.

[2] Stewart, C. P., and White, A. C., *Biochem. J.*, 1925, xix, 840.

In experiment 1, ¼ cc. of adrenalin chloride (Parke, Davis and Company) was injected subcutaneously every one quarter of an hour for 6 hours. Experiment 2 differed from 1 in that the injections of adrenalin were continued for 4 hours only. In experiments 3 and 4, adrenalin injections were also stopped at the fourth hour, but they were followed at that time by the subcutaneous injection of 5 units of insulin per kilo.

TABLE I.
Fat Content of Plasma of Arterial Blood of Dogs. mg. %.

Exp. No.	Days fasted	Before injection	Hours after injection			
			2	4	6	8
1	4	557	978	1431	1668	
2	1	804	974	1196	1258	
3	2	508	526	936	717	462
4	5	543	709	829	859	1120

It will be observed in Table I that subcutaneous injections of adrenalin may give rise to lipemia. Apparently insulin overcame the lipemic action of adrenalin in experiment 3. It is generally known that insulin reduces the lipemia of diabetes. Rony and Ching[3] have shown that insulin has the same effect on post-prandial lipemia.

4985

Effect of Pituitary Extracts on Basal Metabolic Rate.

H. E. HIMWICH AND F. W. HAYNES.

From the Department of Physiology, Yale University, New Haven.

Previous studies on the effect of pituitary extracts on basal metabolic rate have not been conclusive, although an increase has been indicated. The work of Oliver Kamm who succeeded in separating the oxytocic and vasomotor principles of pituitrin, has made it possible to fractionate their effects on metabolism. In the present experiments the metabolic rates of male rats fasted approximately 24 hours, were determined in a Haldane-Pembrey apparatus, before and after the subcutaneous injections of chloretone-free pitocin, pitressin and pituitrin (surgical). The limit of error of the method was considered to be ± 5%.

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[3] Rony, H. R., and Ching, T. T., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 533.

A slight increase in metabolic rate was observed after the injection of pitocin. Doses ranging from 2.5 to 80 units per kilo showed an average rise in the oxygen consumption of 32% above the basal level, during the first hour after injection. Part of this rise, probably about 16%, was due merely to the effect of the injection. The average increase for the second and third hours after injection was 13%. The rise was not directly related to the size of the dose.

Results with small units of pitressin (17-40 units per kilo) were variable. Doses from 50-100 units per kilo decreased the oxygen consumption 22% in the first hour after injection. The average oxygen consumption for the second and third hours did not differ significantly from the basal.

Pituitrin (surgical) produced an average fall of 19% in the oxygen consumption in the first hour after injection and 7% in the 2 succeeding hours. A summary of results is shown in Table I.

TABLE I.
Effects of Subcutaneous Injections of Pituitary Extracts on Basal Oxygen Consumption.

Substance Injected	Number of Experiments averaged	Dose	1st hr. after injection, % of basal	2nd-3rd hr. after injection, % of basal
Sterile water	7	0.5-2.5 cc.	+16	+6
Pitocin	8	10-80 units per kilo	+32	+13
Pitressin	13	17-40 units per kilo	+5	—1
Pitressin	5	50-100 units per kilo	—22	—10
Pituitrin	6	0.5-1.5 cc.	—19	—7

We wish to thank Doctor Oliver Kamm, through whose courtesy Parke, Davis and Company has supplied us with pitocin and pitressin.

4986

Gastric Juice Acidity at the End of the Secretory Period.

FRANKLIN HOLLANDER. (Introduced by I. S. Kleiner.)

From the Department of Physiology, New York Homeopathic Medical College and Flower Hospital.

For many years, the theory of Heidenhain and of Pavlov—that the concentration of acid in the isolated parietal secretion is constant within narrow limits—was almost universally accepted. Most of the recent contributors, however, have taken the view that this fluid is a mixture of HCl and neutral chlorides, in varying proportions. The present paper is a refutation of one of the major arguments advanced by this latter group,[1] and deals with the decrease in acidity of the gastric juice during the latter part of the digestive period.

Variations in acidity of gastric juice are always observable, even in the relatively pure juice obtained from dogs with stomach pouches. Such variations were interpreted by Pavlov as resulting from partial neutralization by mucus. A major point in support of this explanation is the obvious parallelism of acidity and the rate of post-prandial secretion during any one experiment. In a previous communication, such correlation was confirmed[2] for food and small doses of histamine. When the gastric juice was collected by continuous drainage, samples being taken during quarter or half hour periods, it was observed that every increase or decrease in volume per period was accompanied by a like change in acidity. However, after increasing the rate of secretion sufficiently, as by the injection of a large dose of histamine, this parallelism vanished and the acidity became constant at a pH of 0.91. This is entirely in accord with Pavlov's conception.

In opposition to the theory, it was first suggested by Rosemann[3] and by Foster and Lambert[4] that the fluid secreted by the parietal cells possesses a fairly constant chloride content. With increased intensity of stimulation, and therefore increased rate of flow, the proportion of neutral chloride converted to acid is likewise increased, resulting in the commonly observed fluctuations in acidity. One of the chief arguments by Rosemann[5] is: If the low acidities observed at the beginning of the secretory period are due to neutralization of mucus which is present in the pouch at this time, it might be expected that all such mucus would have been removed by the latter part of the experiment. Thus, there should be no corresponding decline in acidity at this time. However, such a reduction does occur, and the only other possible explanation—that of an increase in mucus flow—is not substantiated by a marked increase in mucin content of the last samples of juice.

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[1] Babkin, *Am. J. Surg.*, 1929, vii, 499.

[2] Hollander, *J. Biol. Chem.*, 1927, lxxiv, xxiii.

[3] Rosemann, *Pfluger's Arch.*, 1907, cxviii, 467.

[4] Foster and Lambert, *J. Exp. Med.*, 1908, x, 820.

[5] Rosemann, *Virchow's Arch.*, 1920, ccxxix, 67.

In order to settle the argument centering about this point, numerous experiments have been performed with Pavlov and Heidenhain pouch dogs. Both foods and histamine injections have been employed as stimuli. The gastric juice was obtained by the continuous collection method; i. e., by the use of a soft rubber catheter kept in the pouch of a dog, supported by a sling in an upright position. Precautions were taken to obviate excessive mucus secretion by irritation; also admixture of exudate from the outer abdominal wall with the juice was prevented.

The first series of experiments employed a weak stimulus, so that the secretion at no time attained a pH value as low as 0.91. In such experiments a decrease in acidity is invariably found at the end of the secretory period. However, a comparison of the acidities at the beginning and towards the end of the period (*at times such that the secretory rates are about the same)* indicates that almost invariably the acidity is much lower at the beginning than at the end. Of course, when collection of juice was continued beyond this point, the acidity fell still lower and approached the value for unacidified mucus. Such values are obviously not comparable with the early ones, because of the greatly increased proportion of mucus which they contain.

In the second series, the histamine dose was raised to 0.4 mg. per kg. In all cases, by the second or third quarter-hour sample, the acidity had attained the maximum value where it remained throughout the experiment. Even the last sample collected showed the same acidity—or one below it by an insignificant amount—provided mucin clots were absent. Usually the presence of mucin in these samples betokens an almost complete cessation of acid secretion. Thus by increasing the rate of flow of HCl, the contact surface between mucosa and catheter is lubricated sufficiently to reduce mucus secretion to a minimum. This in turn, minimizes the reduction in acidity of samples collected towards the end of the secretory period.

These observations are wholly in agreement with the theory of Heidenhain and Pavlov. Furthermore, they satisfactorily account for the evidence cited above in opposition to this theory. The agent which is effective in neutralizing the acid is not the mucus which is already present at the beginning of secretion. On the contrary throughout the experiment there is a continuous flow of such neutralizing and diluting fluid, due in great part to irritation of the mucosa by the catheter. With reduction in this mucus flow, the late fall in acidity becomes less and less observable.

4987

Does the Virus of Poliomyelitis Survive in the Monkey Testicle?*

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From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

Among the points of major interest in experimental poliomyelitis is the question as to how long the virus is capable of surviving in different organs. Such data not only furnish information which is helpful in studying the factors responsible for selective tissue susceptibility, but may also add to our knowledge concerning the mode of invasion in the disease. From this viewpoint, the testicle has assumed particular prominence in practically all neurotropic virus diseases. It may either serve as portal of entry from which a generalized infection takes its beginning, or, more particularly, offer excellent ground for the development of a circumscript local lesion (herpes, vaccinia). It appears that infection by the testicular route so far has not been attempted in experimental poliomyelitis, save for the recent work of Thompson,[1] who reported his inability to infect rabbits by intratesticular injection with monkey virus. In these experiments it was also found that the virus does not survive for a period of even 24 hours in the rabbit testicle, as determined by subsequent transfer to the monkey.

We considered it important to extend these experiments to a study of the capacity of the testicle of a susceptible animal, i. e., the monkey, to serve either as a portal of entry for the disease or as a suitable living culture medium for the infectious agent. These experiments moreover afforded an opportunity to determine whether

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* Under a grant from the Milbank Fund for the study of infantile paralysis.

[1] Thompson, R., *J. Exp. Med.*, 1930, li, 777.

intratesticular injection of virus conveyed any active, generalized immunity to the monkey.

A total of 8 Rhesus monkeys, in several experiments, were injected intratesticularly with poliomyelitis passage virus (Aycock strain), the animals receiving doses of 0.5-1. cc. of a 20% monkey virus cord emulsion into each testicle. The virulence of the virus in each case was confirmed by parallel intracerebral inoculation of a control monkey with the same cord emulsion. It is necessary to select somewhat older animals with well-developed testicles for this work since considerable difficulty may be encountered in forcing so much fluid into the organ which is of unusual hardness in the monkey. In some of the 8 monkeys the injected testicles were left *in situ*, and the animals were observed for the occurrence of any symptoms of the disease. With the majority however, the testicles were removed after various periods of time, ranging from 24 hours to 15 days, and tested for presence of virus. For this purpose, the greater part of the fresh organ was ground and emulsified and the emulsion injected intracerebrally (any excess intraperitoneally) into a new monkey, the remaining portion of the tissue being saved for histological examination.

None of the animals infected intratesticularly ever showed any evidence of generalized poliomyelitis during a period of observation extending from 1 to 2 months. We succeeded in one single instance only in recovering potent virus from an infected testicle, which in this case happened to carry virus for a period of 3 days. On repetition, however, 3-day testicles yielded only negative results. In one case in which the inguinal lymph glands were tested they were found free from virus. Neither the original testicle-infected monkey nor the transfer animals which had been injected intracerebrally with the testicle emulsion, had acquired any active immunity, as evidenced by their full susceptibility to subsequent intracerebral re-infection with potent virus.

Histological examination of the various testicles demonstrated an almost complete absence of any characteristic tissue reaction. A slight leucocytic infiltration found in the earlier specimens was duplicated by similar findings in the testicle of a monkey which had, for reasons of control, received an intratesticular injection of normal monkey cord. We could discover no unmistakable evidence of the presence of inclusion bodies in any of the testicles studied particularly for this purpose.

We conclude from the above experiments that infection by the intratesticular route with poliomyelitis virus, is impossible in the monkey and that survival of the virus in this organ is exceptional, even within very short limits of time. With this agrees the absence of any demonstrable specific tissue lesion in the infected testicle. Finally, it is noteworthy that neither the testicle-infected monkeys nor the animals receiving testicular emulsion by intracerebral injection had acquired any active immunity.

4988

The Use of the Stuphenphotometer for Measuring Percentage Hemolysis.

ERIC PONDER.

From Washington Square College, New York University.

The methods for measuring percentage hemolysis may be divided into two classes, (1) subjective methods, such as those in which the observer adjusts the depth of a suspension until some object is just visible, or in which the hemolysing suspension is matched nephelometrically against standards, and (2) methods in which a photosensitive element,such as a potassium or selenium cell, a radiometer, or a thermopile, is used to measure the intensity of light transmitted by the suspension. The methods in the first group are unsatisfactory because too much depends on the judgment of the observer, and because rapid work is usually difficult or impossible; the photometers included in the second group, on the other hand, are usually unsatisfactory because of the difficulty in obtaining sensitivity and stability at the same time when the apparatus is so arranged as to make rapid consecutive readings possible. All methods, moreover, (except the radiometer method) are insensitive to changes in the range of 0% to 10% lysis. Ideally, the method used should be (a) capable of an accuracy of not less than ±2%, even in the range from 0% to 10% hemolysis, (b) sufficiently rapid to allow readings at intervals as short as 5 seconds, and (c) perfectly reliable in the sense that its performance does not depend on factors which require continual control. This last condition virtually rules out any method which is not entirely optical.

These conditions are met by the use of the "Stuphenphotometer" ("Stupho"), a photometer recently designed by Pulfrich of Carl Zeiss, in conjunction with a simple device for containing the cell suspensions whose opacity is to be measured. This

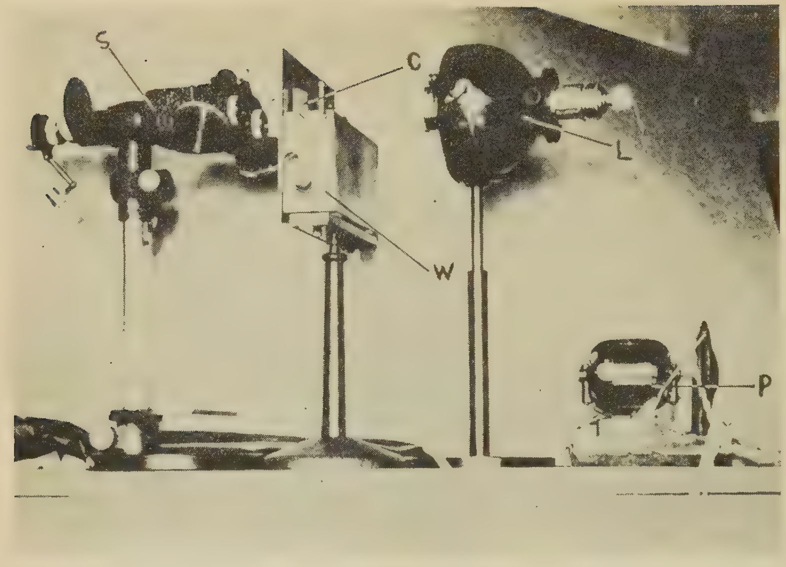

Fig. 1.

L—Stupho lamp; S—Stuphenphotometer; W—Water bath; C—one of the cells containing hemolytic system; P—air pump.

consists of a large electrically heated and thermostatically controlled water-bath, one side of which is prolonged upwards as a screen, and pierced with two holes, 1.6 cm. in diameter, which exactly correspond to the 2 circular openings of the "Stupho" which faces them. (See Fig.) Immediately behind one of these holes is a glass cell, 3 mm. x 2 mm. x 0.5 mm., while behind the other is a row of 3 similar cells, any one of which can be brought opposite the hole by turning a handle. The cells are arranged, moreover, so that the hole in the screen is completely covered when they contain 2.0 cc. of fluid, all light passing from the Stupho lamp through the hole into the Stupho first passing through the fluid; their total capacity, however, being 3.0 cc., additions to the hemolytic systems, of initial volume 2.0 cc., can be made at any time. The hemolytic systems are kept stirred by very fine jets of compressed air, delivered from a tiny pump. The water bath and cells are also provided with a cover, not shown in the figure.

The range of light intensity over which the Stupho works is enormous (2 intensities differing a thousand-fold can be matched quite easily); the whole range of hemolysis, from 0% to 100%, can, therefore, be measured with a single standard. This may be an unhemolysed suspension of "standard" strength (10^8 cells per 2 cc. of the hemolytic system), but is preferably a permanent standard made by enclosing a piece of pink paper between 2 sheets of glass; this standard is then placed against the face of the single chamber which covers one of the two holes, while the chamber itself is filled with distilled water. A green filter, which enables very accurate readings to be made by the dark adapted eye, is used in the Stupho eyepiece. A series of readings for suspensions showing known degrees of lysis are given below, the right-hand drum, corresponding to the permanent standard, being set at 50. For each degree of lysis is shown (1) the reading of the Stupho left-hand drum, and (2) the greatest error made in a set of 10 successive readings, the error being expressed as a percentage of lysis. In the case of all readings, only 2 seconds was allowed for matching the fields of the Stupho; the table accordingly shows the precision of the instrument when working under this exacting condition.

TABLE I.

Lysis, %	Reading	Greatest Error in 10 Readings
0	82.1	1.5
10	68.5	2.4
20	56.4	2.5
30	44.0	3.0
40	33.0	2.2
50	23.5	2.3
60	16.1	2.0
80	7.3	1.9
100	1.44	1.8

The greatest error made in any single reading, when expressed as a percentage of lysis, thus never exceeds 3%, and further, the standard error of the successive readings is always found to be less than ±0.5%; the precision of the method thus exceeds any used hitherto. Even were a smaller precision attained, however, the method would be much more useful in practice than existing methods by reason of its extreme simplicity.

4989

Sub-Threshold Hyperglycemia and Glycuresis.

U. J. SALMON. (Introduced by Sidney Bliss.)

From the Department of Biochemistry, McGill University, Montreal, Canada.

Folin[1] states that "Glycuresis is independent of the level of blood sugar and is not normally obtained from the ingestion of pure glucose."

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[1] Folin, O., and Berglund, H., *J. Biol. Chem.*, 1922, li, 213.

The opposite view is expressed by Benedict in the following:[2] "We note at once that in every instance there is a definite general form to the curve of the sugar elimination following the ingestion of the sugar. The general form of this curve is *a rise in the middle and a fall at both ends,*" and " . . . offers convincing evidence that the experimental factor (glucose ingestion) was responsible for an increased urinary sugar excretion in these experiments."

The experiments reported were designed to compare glycuresis during short intervals, with frequent values for blood sugar for comparison.

Thirty-six experiments were performed on 25 human subjects. We have found that the moderate rises in the blood sugar level after the ingestion of either pure glucose or a mixed meal are accompanied by parallel increases in the amount of urinary sugar excreted per hour. With the ingestion of 50 gm. of pure glucose in 20% solution we have observed an increase in urinary sugar from 14 mg. per hour to 26 mg. and 60 mg., with a return to 16 mg. and 15 mg. per hour, and this curve of increased sugar elimination follows closely the fluctuation in the level of the blood sugar, which in this case went from 80 mg. to 85 and 89 and then returned to 83 and finally 79 mg. per 100 cc. of blood, all sub-threshold values.

Similar results were obtained after mixed meals.

The method employed for the estimation of blood and urinary sugar was that of Folin[3] with the modification for urinary sugar determination outlined by Folin and Svedberg.[4]

While this method is not specific for glucose, the results obtained with it more nearly express the true sugar content of blood than older methods. We have recognized the value of doing similar experiments by methods capable of measuring the true glucose content of blood and urine, and such experiments are now under way and will be reported in the near future.

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[2] Benedict, S. R., and Osterberg, E., *J. Biol. Chem.*, 1923, lv, 769.

[3] Folin, O., *J. Biol. Chem.*, 1926, lxvii, 357.

[4] Folin, O., and Svedberg, A., *J. Biol. Chem.*, 1926, lxx, 405.

4990

Lethal Factors in Experimental Infections of Trypanosoma equiperdum in Rats.*

JUSTIN ANDREWS AND CARL M. JOHNSON. (Introduced by W. H. Howell.)

From the Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland.

A biochemical and pathological study of experimental *Trypanosoma equiperdum* infections in laboratory-bred rats has disclosed that the animals died with a significantly reduced blood pH, lowered alkali reserve, and greatly diminished blood oxygen content, i. e., they finally succumbed to a condition of non-volatile, uncompensated acidosis. These findings are in harmony with the previously reported excessive lactic acid content and reduced oxygen content of the blood of trypanosome-infected rats (Kligler and Geiger;[1] Kligler, Geiger and Comaroff[2]). A progressive hypoglycemia accompanied these terminal manifestations as has been demonstrated by a number of investigators.

Tissue changes found at death were pronounced vasodilatation and congestion throughout the body, extreme enlargement of the spleen, varying degrees of pulmonary edema and occasionally emphysema, distension of the right heart and engorgement of the coronary vessels. The most significant development was the formation of intravascular blood clots which were most prominent in the left side of the heart, but which were also found in the right heart and the vessels of the lungs. These clots were not post-mortem clots nor were they typical laminated thrombi. They were composed of red cells in a mesh of fibrin enclosing an agglomeration of trypanosomes.

It is believed by the authors that the obstruction to the circulation occasioned by these blood clots results in pulmonary edema which leads progressively to the manifestations of dyspnea, suffocation, and finally asphyxia. These changes account for the terminal syndrome, viz., convulsions, almost total lack of oxygen and excessive amounts of lactic acid in the blood, lowered alkali reserve and subsequently reduced pH of the blood. The reduction of blood

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* This work has been aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Kligler, I. J., and Geiger, A., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 229.

[2] Kligler, I. J., Geiger, A., and Comaroff, R., *Ann. Trop. Med. and Parasit.*, 1929, xxiii, 325.

sugar is tentatively explained as follows: the utilization of glucose by the rapidly multiplying trypanosomes exercises a progressively increasing demand upon the glycogenolytic function of the liver. The release of glycogen by the liver is probably facilitated under the existing condition of reduced alkali reserve. This may account for the occasional temporary hyperglycemia noted and for the increase in muscle glycogen (Bruynoghe, Dubois, and Bouckaert[3]). It seems probable that the central necrosis of the liver, caused by the anoxemia, interferes with the glycogenic function of that organ, so that it ultimately fails to supply sufficient glycogen to maintain the normal level of glycemia.

4991

The Passage of the Spermatozoa and Ova Through the Oviducts of the Rabbit.*

G. H. PARKER.

From the Zoological Laboratory, Harvard University.

In earlier papers[1, 2, 3] I pointed out that the oviducts of the turtle and of the pigeon possess narrow pro-ovarian tracts of cilia by which spermatozoa may be carried from the lower part of these ducts to the neighborhood of the ovary. It was a matter of interest to ascertain whether such tracts obtain in the mammals. For this purpose I have studied the oviducts of the rabbit.

In the female rabbit the vagina is a single median tube about 5 cm. long. This is followed by two independent uteri each about 7 cm. long, opening separately into the vagina. From each uterus a narrow Fallopian tube approximately 7 cm. long is continued forward to its termination in an infundibulum close to the ovary of its own side. After coition spermatozoa reach the anterior end of the uterus in about 2 hours and are found at the infundibulum in 2 hours more. Ovulation takes place about 10 hours after coition. Spermatozoa will live in the female ducts of the rabbit for about 30 hours, the eggs

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[3] Bruynoghe, M., Dubois and Bouckaert, *Bull. L'Acad. Roy. de Med. de Bel.*, 1927, v, 7, 142.

* The expense of these investigations was borne in part by funds received from the National Research Council, Committee for Research Problems of Sex.

[1] Parker, G. H., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 52.

[2] Parker, G. H.,, *Am. J. Physiol.*, 1926, lxxxvii, 93.

[3] Parker, G. H., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 704.

after liberation from the ovary, for from 2 to 4 hours. It therefore follows that spermatozoa which can live about 30 hours take in all 4 hours to reach the spot where 6 hours later eggs that can live from 2 to 4 hours will arrive. The questions that I wish to consider are how these elements, male and female, transferred through the rabbit's ducts.

The spermatozoa are deposited by the male rabbit in the vagina of the female and if the female is killed and examined as quickly after coition as possible, living spermatozoa will be found in the lower part of the uterus. The period between coition and examination is so brief, 3 or 4 minutes, that it is impossible that the spermatozoa through their own activity could reach so high a position in the oviducts as the lower part of the uterus. It is, therefore, probable that these cells are transferred from the vagina to the uterus by some activity of the female. This region of the uterus is ciliated and the cilia beat toward the exterior. They, therefore, do not afford an easy means of transfer for the sperm cells. If artificial insemination is performed on a female rabbit slightly under the influence of ether and so guarded as not to suffer an orgasm, the spermatozoa will remain for over an hour in the vagina without making their way into the uterus. In consequence of these conditions it is concluded that the transfer of the relatively voluminous sperm from the vagina to the uterus takes place through a muscular response of the oviduct in the nature of an antiperistalsis and probably associated with coition.

The ascent of the spermatozoa through the uterus has been attributed in part to the muscular movements of this organ and in part to its scanty ciliary supply. If in a quiescent uterus, freshly removed from a rabbit and kept in Ringer's solution at body temperature, living spermatozoa are injected into the distal end of this organ, they will be found to have arrived at its proximal end in about one hour. Similar injections at the proximal end are followed by spermatozoa at the distal end in about the same time. As such uteri were quiescent it is concluded that the spermatozoa make their way through these ducts by their own powers of locomotion. The spermatozoa are known to swim forward at an approximate rate of 60 μ's per second. They might, therefore, cover the length of the uterus, 7 cm., in 20 minutes. Hence one hour is not an unreasonable time for this migration. The conclusion that the spermatozoa make their way through the uterus by their own powers of locomotion is in harmony with the observation of Lim and Chao[4] that the operational inversion of a section of the uterine duct does not prevent pregnancy.

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[4] Lim, R. K. S., and Chao, C., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 668.

A most thorough examination of the Fallopian tubes in the rabbit has failed to disclose any other form of ciliary action than that of the abovarian type. No tract of pro-ovarian cilia was anywhere discoverable and the tubes in this respect are unlike those in the turtle and in the pigeon. The cavity of the Fallopian tubes in the rabbit is much divided by folds, as in most mammals, and the cilia covering these folds appear in all cases to have an effective stroke away from the ovary. It has been customary to assume that the spermatozoa pass through these tubes by swimming against the ciliary current, thus exhibiting a true rheotaxis.

When a freshly prepared tube is cut open so that its ciliary surfaces can be inspected under the microscope and it is flooded with a mixture of Ringer's solution and spermatozoa, the movements of the sperm cells in relation to the cilia can be easily observed. Many spermatozoa attach themselves by their heads to the surface of the ciliated folds. Their tails trail out from the point of attachment in the direction of the ciliary current. They are, therefore, oriented with their heads up the tube. Should they swim further they would, at least in the beginning of their course, ascend the tube. Such preparations have been watched for a long time but in no instance have spermatozoa been seen to free themselves and swim against the current. In the current next the cilia spermatozoa, swimming indiscriminately in all directions, are carried down the tube. Still further away from the cilia it is usual to find a counter current in which spermatozoa, again swimming in all directions, are being carried up the tube. The cavity of the tube is so generally divided by the folds already mentioned that counter currents are as common as downward currents. This interplay of downward currents and counter currents in the tube results in the transfer of small bodies such as spermatozoa both up and down the tubes. If India ink mixed with Ringer's solution is injected in small amount into the middle of the length of a Fallopian tube, it will sooner or later find its way out of this tube both into the uterus and into the region of the ovary. When an especially thin-walled portion of the Fallopian tube without having been cut open is inspected under the microscope, currents and counter currents can be easily seen in its interior. It is, therefore, concluded that overlapping counter currents are responsible for the transfer of the spermatozoa toward the ovary. This does not imply that the spermatozoa may not move upward under their own locomotion, but it is probable that this method of ascent is much less important than that of the overlapping counter currents. These currents also offer an explanation for the transfer to the region of the ovary of other small bodies such as fragments of epithelium from the uterus, which may thus gain an appropriate position to produce the pathological state of chocolate cysts.

The eggs of the rabbit are discharged from its ovary about 10 hours after copulation. The extrusion of the egg from the follicle is a relatively slow process. While this is going on the infundibulum is, according to Westman,[5] closely approximated to the ovary and the newly liberated eggs are carried into the Fallopian tube in large part by peristaltic suction. As is well known, the eggs pass rather rapidly through the upper part of the tube but much more slowly through the lower part. Much difference of opinion exists on the question of the ciliary and the muscular efficiency of the tube in this transportation. That the Fallopian tube exhibits peristalsis is well established. That in most mammals the tube is lined with cilia that beat toward the uterus is also well known. In certain forms, as, for instance, the mouse, the cilia are partly deficient and in regions where there are no cilia the action of the tube must be purely peristaltic. If the egg of the rabbit is measured it will be found to have a maximum diameter of about 0.16 mm. Its size is such that it cannot pass down the spaces in the Fallopian tube without pressing rather firmly against the faces of the folds in the tube. Since these faces are ciliated it follows that the pressure of the egg against them must bring the cilia into most effective play for the downward passage of the egg. It, therefore, appears probable that in the rabbit the passage of the egg through the tube is dependent in part upon ciliary action and in part upon peristalsis. This conclusion agrees with the observations of Kue and Lim[6] to the effect that when a section of the tube in the pig is reversed sterility follows.

The eggs of the rabbit develop into embryos in the uterus. Observations on gravid females made by the window method show that the discharge of these embryos is accomplished by peristalsis in which the 2 uteri act more or less independently.

Conclusions: 1. Spermatozoa are transferred from the vagina of the rabbit to its uterus by the muscular activity of the duct; 2, spermatozoa pass up the uterus by their own locomotion; 3, they ascend the Fallopian tube possibly by their own locomotion but chiefly through transportation by counter currents induced by the cilia; 4, the egg, in consequence of its size, so presses upon the ciliary surfaces of the tube that the cilia become effective in the downward passage of this body and thus supplement peristalsis.

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[5] Westman, A., *Skan. Arch. Physiol.*, 1926, xlix, 243.

[6] Kue, Y. P., and Lim, R. K. S., *Chinese J. Physiol.*, 1928, ii, 389.

4992

A General Test for Carbohydrates.

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A number of tests for carbohydrates depend upon interaction in the chromogenic system, phenol-aldehyde-acid. The Molisch, the Seliwanoff, the Bial, and the Tollen tests belong to this reaction system. The Molisch reagent employes the phenol, α-naphthol, the Seliwanoff reagent resorcin, the Bial reagent orcinol, and the Tollen reagent phloroglucin. The aldehyde in the reaction system is either the carbohydrate itself or the furfural obtained by the decomposition of carbohydrate by means of more or less concentrated acid. Either the reagent or the final reacting mixture contains hydrochloric or sulphuric acid. The aldehyde and the phenol react to form a compound of chromogenic power. The Seliwanoff, the Bial and the Tollen reagent serve to detect certain types of carbohydrates, while the Molisch reagent is universal in its ability to detect carbohydrates of any number of carbon atoms, aldehydic or ketonic in nature, with or without an open carbonyl group, free or in combination with other compounds.

We have developed a test for carbohydrates, which like the Molisch is of general application, by introducing thymol as the phenol in the system, phenol-aldehyde-acid. We have found that a 5% thymol in 95% alcohol serves as a useful, sensitive and practical reagent for carbohydrates. The thymol reagent has the advantage over the Molisch reagent, since the former is a colorless solution and does not deteriorate on long standing. We have kept the alcoholic thymol solutions in ordinary bottles exposed to window light for over a year without the development of any color.

To make the test we proceed as follows:* 3 or 4 drops of the 5%

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* Molisch described a test for sugar in the urine based upon a reagent containing 15% thymol in alcohol. At this concentration 3 to 4 drops of the solution alone will yield a colored ring with concentrated sulphuric acid. The thymol used in our experiments was obtained from Merck and was of reagent quality.

thymol solution are added to the test solution. A volume of concentrated sulphuric acid is added equal to that of the sugar-containing solution. The acid is carefully poured down the side so as not to mix. A distinct and deeply colored ring appears at the junction of the light test solution and the heavy bottom layer of sulphuric acid. The usual color is a deep red or heavy pink red. Very often there is a purplish tinge mixed in with the usual color.

The reaction is positive with pentoses, hexoses, dihexoses, trihexoses, polysaccharides, glucosides, gums, glycoproteins, glycolipins, nucleic acid, which yields carbohydrate on hydrolysis.

Neither thymol or the Molisch reagent is specific for carbohydrates, since in the reaction system, phenol-aldehyde-acid, aldehydes themselves respond. Positive reactions have been obtained with formaldehyde, paraformaldehyde, acetaldehyde, paraldehyde, chloral hydrate, glyoxylic acid and furfural. Ketones may be substituted for aldehydes in the system.

Many straight chain carboxylic acids are aldehydogenic under the influence of a concentrated acid like sulphuric acid. These acids, therefore, give tests with the thymol reagent as well as with the Molisch reagent. Among the acids that give reactions may be mentioned lactic acid, maleic acid, tartaric acid, aspartic acid, citric acid and oxalic acid.

Neither the thymol nor the Molisch test can be used specifically for sugar in the urine, since certain aldehydes, ketones and organic acids likely to be present give positive reactions. Formic acid, lactic acid, oxalic acid, acetaldehyde, acetone, diacetic acid, and hydroxybutyric acid give color reactions with both reagents.

The sensitivity of both the thymol and the Molisch reagent is very great. With both reagents glucose yields the characteristic reaction in 0.001% solution, sucrose in 0.0001% solution, and raffinose in 0.00005% solution.

4993

A Test for Cholesterol Based Upon the Use of Sulphuric Acid Containing Selenious Acid.

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Cholesterol dissolved in chloroform gives a characteristic color test with concentrated sulphuric acid containing in solution 125 mg. of sodium selenite for every 25 cc. of acid. The test is made by adding to a chloroform solution of cholesterol an equal volume of the selenious-sulphuric acid reagent. The reaction is positive when the top layer assumes a deep beautiful purple, while the lower layer assumes a red brown to a very dark brown color, but does not show any green fluorescence.

The test is more sensitive than the Salkowski test. The chloroform solution containing 0.0025% of cholesterol does not yield the typical color layers observed with the concentrated sulfuric acid in the Salkowski test, which is concentrated sulphuric acid. Such solution does not give the cherry red color in the top layer, and does not give in the bottom layer a red color by direct light and a green fluorescence in transmitted light. Instead it yields only a faint trace of pink in the top layer, and in the bottom layer a slight yellow color in direct light and a slight green fluorescence by transmitted light. On the other hand, the same strength solution of cholesterol with an equal volume of the selenious-sulphuric acid reagent still gives a deep purple in the top layer, and in the bottom layer a reddish brown without fluorescence.

With a 0.001% cholesterol solution the reaction is still positive with the new reagent, while with the sulphuric acid in the Salkowski test the reaction is atypical, no color being present in the top layer and but a very faint yellow color in the bottom layer, which by transmitted light gives a very slight green fluorescence. A 0.0005% cholesterol solution does not yield anything typical or atypical with both reagents.

A mixture of sulphuric acid with tellurous acid proved ineffective owing to the insolubility of the tellurium compound in the acid. A suspension of sodium tellurite was used containing 125 mg. per 25 cc. of acid. When mixed with an equal volume of cholesterol solution the upper layer was colored red to orange or orange brown, while the lower layer was of reddish brown to light orange with a greenish fluorescence in transmitted light. The lower layer after a while became white, due to the settling of the insoluble tellurium compound. On the whole it may be stated that no advantage was gained by the addition of the tellurium compound to the concentrated sulphuric used as the reagent to test for cholesterol.

4994

A Test to Differentiate Ergosterol From Cholesterol.

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When sulphuric acid or a mixture of selenious and sulphuric acids is used as a reagent for cholesterol dissolved in chloroform, the two layers, that is the chloroform and the acid layer, assume different colorations. With sulphuric acid alone the chloroform layer is cherry red and the acid layer shows green fluorescence. With the selenious-sulphuric acid mixture, the chloroform layer assumes a deep beautiful purple, while the lower layer is red brown but does not display green fluorescence.

When, however, ergosterol is used with either reagent, the acid layer is colored *but the chloroform layer remains free from color.* With concentrated sulphuric acid and an equal volume of chloroform solution, the acid layer ranges in color from a brownish red in a 1%, to cherry red in a 0.5%, to light cherry red in a 0.1%, to orange in a 0.04%, and to yellowish orange in a 0.025% solution of ergosterol. In all these concentrations the typical green fluorescence is also observed in the acid layer.

With concentrated sulphuric acid containing 125 mg. of sodium selenite per 25 cc. of acid, the chloroform layer ranges in color from dark red brown to light red brown in the above concentrations of ergosterol while no green fluorescence is noted. *The chloroform layer remains uncolored.*

From the above results the conclusion may be drawn that cholesterol on treatment with acid forms a decomposition product or products giving rise to colored solutions in chloroform and in sulphuric acid or in both. Ergosterol, on the other hand, seems to give a decomposition product or products dissolving with color formation only in sulphuric acid.

Solutions of cholesterol in chloroform remain colorless, while solutions of ergosterol rapidly assume a deep yellow-brown tinge. Irradiated ergosterol develops the color more rapidly and more intensely than the non-irradiated sterol.

4995

Observations on the Transmissibility of Lymphoid Leucemia of Mice.*

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Two spontaneous cases of lymphoid leucemia of mice have been observed in a stock of about 300 mice (over 8 months of age) designated as strain A. The systemic enlargement of all superficial lymph nodes drew attention to this condition. The white blood count was 157,000 in one case and 315,000 in the other; in both, the spleen was very much enlarged and extensive lymphomatous infiltrations were found in the liver. Both cases have been successfully transmitted to normal mice of the same strain and of another strain named R. In about 800 mice of the latter strain, which are still alive, and in about 100 autopsied cases of the same strain (above the age of 8 months) no leucemia or lymphosarcoma has been found. Table I is a summary of the first 7 transmissions including first passages from the spontaneous cases and 2 second, one third and one fourth passage from the first case, and one second passage from the second case.

TABLE I.

	Number of mice inoculated	No. of cases of leucemia	No. of lymphosarcomas
Mice of strain A injected intraperitoneally	70	1	1
Mice of strain A injected subcutaneously	16	0	0
Mice of strain A injected intravenously	99	12	1
Mice of strain R injected intravenously	73	10	2

Lymph nodes or lymph nodes and spleen from leucemic mice were cut up with a small scissors and Locke solution was added drop by drop. After filtration through a small piece of cotton the turbid solution containing about 40,000 to 150,000 lymphocytes per cm. was injected in amounts of 0.1 to 0.2 cc. The blood of a leucemic mouse, injected into the tail veins of 5 mice caused leucemia in 3 and lymphosarcoma in one.

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* This investigation has been supported by a Fund for the Study of Leucemia and Related Diseases.

The incubation period of the instances of leucemia under observation varied between 14 and 33 days, the average being 26 days. The leucocyte count of the transmitted cases at the height of the leucemia varied between 239,000 and 570,000.

The older literature on attempts to transmit leucemia has been reviewed by Opie.[1] More recently Korteweg[2] succeeded in transmitting "leucosarcoma" from mice to a non-inbred stock of mice by subcutaneous and by intraperitoneal injections. Lymphosarcoma developed in about 25% of the inoculated animals, and most of these showed a terminal leucemic blood picture. Richter and McDowell[3] have failed to transmit leucemia to 145 mice of several strains in which leucemia had not appeared spontaneously, but they found that young mice of a highly inbred leucemic strain, the majority of which would develop the disease at a later age, developed it rapidly when inoculated intraperitoneally or subcutaneously.

The data presented above show that by intravenous inoculation and perhaps also occasionally by intraperitoneal injections leucemia is transmissible even to mice of a strain in which leucemia has not been known to occur.

When the onset of the experimental leucemia was closely watched a systemic enlargement of the lymph nodes was the first change observed. A characteristic hyperplasia of the lymphoid tissues apparently precedes leucemia, even if the leucemic cells are introduced into

TABLE II.

Marked lymphoid hyperplasia			Subsequent blood examinations
No. of days after inoculation	White blood count	% lymphocytes	
14	23,000	65	After 20 days† W.B.C. 141,000, 81% L.
19	17,000	41	" 24 " W.B.C. 50,000, 68% L.
			" 29 " W.B.C. 219,000, 94% L.
25	27,000	71	" 28 " W.B.C 150,000.
16	8,000	46	" 23 " W.B.C. 146,000, 79% L.
16	19,000	68*	" 23 " W.B.C. 229,000, 83% L.*
21	13,000	79	" 27 " W.B.C. 32,000, 84% L.
			" 29 " W.B.C. 125,000, 87% L.
19	21,000	30	" 26 " W.B.C. 158,000, 83% L.

* One mitotic figure. † After inoculation.

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[1] Opie, E. L., *Medicine*, 1928, vii, 31.

[2] Korteweg, R., *Z. f. Krebsf.*, 1929, xxix, 455.

[3] Richter, M. N., and McDowell, E. C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 362; *J. Exp. Med.*, 1930, li, 650.

the circulation of normal mice. Table II illustrates these observations.

The experience gained in study of the transmissible leucemia of mice suggests that leucemia begins with a tumor-like proliferation of lymphoid cells. Mice of any age may succumb to leucemia if leucemic cells are introduced into their body. The malignant lymphoid cells, whenever they enter the circulation, lodge and multiply chiefly in the lymphoid tissue, the blood stream being invaded only after a certain degree of hyperplasia has been attained.

4996

Effects of Hypophyseal Hormones Upon Amblystoma Larvae, Following Transplantation or Injection, With Special Reference to the Gonads.

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When the hypophysis of an adult western axolotl is transplanted intact into young larvae of *Amblystoma trigrinum*, 30-35 mm. in length, the graft usually establishes itself permanently. The results are soon manifested in the following order: (1) the pigmentary system responds by a gradual expansion of the melanophores and an increase in number culminating in sooty blackness; (2) a marked growth stimulation with acromegalic symptoms; (3) a hypertrophic effect upon the immature gonads, which is relatively enormous and occurs without exception in the case of the testis, but is relatively slight and frequently absent in the case of the ovary. There is a tendency to metamorphose earlier than normals reared under identical conditions of environment and nutrition.

The pigmentary reaction begins several days after implantation, and first appears as an intensification of the dark areas in the normal pattern. Soon, however, darkening of the lighter areas begins, and in 2 to 3 weeks a uniform blackness of sooty intensity is attained. This effect is attributable to the hormone produced by the pars intermedia, and is well known from the work of many, among whom may be mentioned Smith and Smith,[1] and Allen.[2] The effect upon growth is seen in a tendency to slightly greater length, greater girth, and a conspicuously enlarged head in which the ratio of width to length is noticeably increased. The growth effect and acromegalic condition are attributed to the hyperstimulative action of the pars anterior. This stimulation of growth is, conversely, to be expected from the work of Allen[3] and Smith,[4] both of whom observed retardation of growth following hypophysectomy in frog tadpoles. Also, Evans and Long[5] produced gigantism in rats by administration of an alkaline extract of the mammalian anterior hypophysis. But so far as the writer is aware, acromegaly has not previously been produced experimentally in amphibians.

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[1] Smith, P. E., and Smith, I. B., *Endocrinol.*, 1923, vii, 579.

[2] Allen, B. M., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 504.

The most striking effect is upon the gonads of the male. The ovaries show little or no response to this treatment. Of 7 experimental animals, 3 were males, and one killed 55 days after implantation already showed a very marked enlargement of the testes and a more advanced histological structure. The other 2, preserved after 105 days, when just entering upon metamorphosis, showed swelling of the cloacal lips, extreme enlargement of the testes with increase in volume of 600%, and almost mature spermatozoa, at a total age of 140 days. That the testes of larval Amblystoma are selectively affected is unexpected and at variance with all results obtained in mammals, where the ovary is far more responsive (the work of Smith and Engle[6] is typical).

When the procedure is varied by injection of an alkaline extract of the anterior lobe of the mammalian hypophysis, the same results obtain. In 8 experimental larvae, injected 12 times over a period of 30 days and killed at an age of 75 to 80 days, 5 proved to be males. All were positive. The least effect showed a volumetric increase of the testis of more than 200%, while the best responses were of the same degree as after implantation, although, as the animals were considerably younger, spermatogenesis was not so far advanced. In every case cloacal enlargement, a secondary sex character of the male, was marked. Thus out of 15 experimental animals implanted or injected, 8 proved to be males, and all responded without exception with a volumetric increase, varying according to age and treatment from somewhat more than 200% to more than 600%. This growth response was accompanied in the extreme cases by advanced spermatogenesis and appearance of secondary sex characters in larvae not yet metamorphosed. As stated, the effect upon the ovary is relatively insignificant.

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[3] Allen, B. M., *Anat. Rec.*, 1916, xi, 486; *Biol. Bull.*, 1917, xxxii, 117.

[4] Smith, P. E., *Anat. Rec.*, 1916, xi, 57.

[5] Evans, H. M., and Long, J. A., *Anat. Rec.*, 1921, xxi, 62; *Proc. Natl. Acad. Sci. Wash.*, 1922, viii, 38.

[6] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927-1928, xl.

Riddle and Flemion[7] administered fresh adult hypophysis and various extracts of beef hypophysis to young pigeons and obtained what was probably pronounced growth of the testes. However, the extreme range of variation of the controls and the very irregular response of the experimental individuals make it very difficult to estimate the degree of response or to be sure whether the response is specific. This is the only work known to the writer with results comparable to those reported here. Series of experiments are now in progress in which the parts of the hypophysis are transplanted separately, and different extracts of the hypophysis are injected.

4997

Development and Fate of Spermatozoa in the Epididymis and Vas Deferens in the Guinea Pig.

WILLIAM C. YOUNG AND FIORINDO A. SIMEONE.
(Introduced by A. M. Banta.)

From the Arnold Biological Laboratory, Brown University.

It is well known that spermatozoa in mammals attain what appears to be their full structural development while contained in the seminiferous tubules, but that they are not in a position to be discharged until they have passed through the long coiled ductus epididymidis and have reached the posterior end of the vas deferens.

There is no agreement as to the significance of this long passage through the epididymis. In earlier papers (Young[1, 2]), the suggestions were made: First, that the epididymis provides an environment in which spermatozoon development, not complete when these cells leave the testis, can continue until functional maturity has been attained, and (2) that once spermatozoa become mature, there is no influence which preserves them indefinitely in a condition for effecting fertilization.

Because this general idea has not been expressed previously, except possibly as isolated statements by different writers might be pieced together, supplementary experiments seemed desirable. In addition, the suggestion that spermatozoa age and become incapable of functioning interested us in the nature of the mechanism by which a constant supply of viable spermatozoa is maintained at the posterior end of the vasa deferentia in males which are not allowed to mate.

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[7] Riddle, Oscar, and Flemion, Florence, *Am. J. Phys.*, 1928, lxxxvii.

[1] Young, W. C., *J. Morph. and Physiol.*, 1929, xlvii, 479.

[2] Young, W. C., *Ibid.*, xlviii, 475.

The suggestion that the epididymis is an organ in which spermatozoa may at one time be maturing and may at another time be aging was tested by 2 experiments.

In the first, 68 females selected during the oestrum were inseminated with spermatozoa removed from the proximal ends of the epididymides from normal males, and 69 were inseminated with spermatozoa removed from the distal ends of the same epididymides. The percentage of fertile inseminations was 32.4 in the former group and 71.0 in the latter group. The difference is sufficient to indicate the greater functional capacity of spermatozoa from the distal end.

In the second experiment this procedure was modified. The head of the epididymis and the vas deferens were ligated in such a manner that spermatozoa could neither enter the epididymis from the testis nor be discharged into the urethra. After 25 days spermatozoa from the proximal ends were used for inseminating 81 females and spermatozoa from the distal ends were used for inseminating 85 females.

If it is true that spermatozoa contained in the epididymis attain an optimum functional capacity and then age and become incapable of functioning, it would be expected that the younger spermatozoa confined in the anterior end would show an increased capacity for effecting fertilization and the older spermatozoa confined in the posterior end would show a decreased capacity for effecting fertilization.

The expectation was confirmed. The percentage of fertile inseminations with spermatozoa from the proximal ends rose to 53.1 and the percentage of fertile inseminations involving spermatozoa from the distal end decreased to 27.1.

This tendency of spermatozoa contained in the epididymis to age and become incapable of functioning suggests, (1) the existence of a constant current of spermatozoa through the epididymis even in the absence of copulations, and (2) the constant removal of old spermatozoa and their replacement by younger spermatozoa.

Both suggestions have been confirmed. When those males in which the heads of the epididymides and the vasa deferentia had been ligated were examined 25 days later, the confined spermatozoa were found tightly packed in the distal end of the tubule; a condition which must be attributable to neuro-muscular action. Any pressure of fluid from the seminiferous tubules is precluded by the ligature of the epididymis head.

The process by which non-discharged spermatozoa are constantly being removed from the posterior end of the vas deferens would appear to be quite different from that which other investigators have described.

We have found no appreciable elimination in the urine as Oslund,[3] and others have claimed. Careful examinations twice daily were made of the urine collected from 5 males over a period ranging from 10 to 46 days without finding more than one or two spermatozoa. We noted the loss of spermatozoa from the epididymides of 4 males without finding more than one or two spermatozoa in the urine during the period of spermatozoa disappearance. Lastly, many other examinations of urine were made under varied conditions without finding more than an occasional spermatozoon.

No evidence for a phagocytosis of spermatozoa such as has been described by Morgenstern,[4] Nemiloff,[5] and Wegelin,[6] could be found in examinations of normal animals.

Careful examinations of the seminal vesicles revealed no disintegration of spermatozoa in this organ such as Königstein[7] has described.

What was found is that there is a continuous dissolution of spermatozoa particularly in the extreme posterior end of the vas deferens. Thousands of isolated, degenerate spermatozoa can be found in dilute suspensions. More striking are the numerous large, spindle-shaped to spherical masses of degenerate spermatozoa from the extreme distal end of the epididymis to the ejaculatory duct. These masses are larger and more numerous in the posterior end of the tract than they are a short distance anteriorly and they provide ample evidence for the wholesale destruction of spermatozoa which is occurring. Inasmuch as spermatozoa may become degenerate before they have passed into that part of the vas deferens which lies in the body cavity, we suggest that age may be a causal factor. It seems not unlikely, however, that the higher temperature encountered in the posterior end of the vas deferens may be a factor superimposed upon that of age.

As an outcome of the experiments described above we believe that a fairly complete account of spermatozoa history from the time they leave the testis until they have reached the posterior end of the vas deferens can be given for at least one species. Spermatozoa are believed to be carried out of the testis into the ductus epididymidis while they are functionally immature. Much of the time consumed in passing through this 9 to 9½ foot tubule is necessary for the completion of their development. Once this is attained, however, there is no influence which preserves them in an optimal functional condition and they begin to age and ultimately to become liquified. There seems to be no general escape from the posterior end of the tract except during copulations and through dissolution.

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[3] Oslund, R. M., *J. Am. Med. Assn.*, 1928, xc, 829.

[4] Morgenstern, Z., *Virchow's Arch. f. path. Anat. u. Physiol.*, 1924, ccl, 648.

[5] Nemiloff, A., *Zeitschr. f. ges. Anat.*, 1926, lxxix, 1.

[6] Wegelin, C., *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1921, lxix, 281.

[7] Königstein, H., *Wiener klin. Wochenschr.*, 1924, xxxvii, 26.

To what extent these observations can be duplicated in other mammals, particularly those which are producing spermatozoa continuously, we do not as yet know.

4998

Biological Effects of Temperature Variations With High Frequency Oscillations.

JEAN MAC CREIGHT AND G. MURRAY MC KINLEY.
(Introduced by H. H. Collins.)
From the Zoological Laboratories of the University of Pittsburgh.

Since there is at present a premature trend toward the use of the vacuum tube high frequency generator in therapeutics, a study of certain extreme local heating effects in animals seems advisable. The following experiments were conducted in the hope of throwing some light on the exceedingly high temperatures generated in local tissues of which the general body temperature gives little or no warning.

When week old Albino rats were exposed to an electrostatic field obtained by an apparatus giving a frequency of 100,000,000 cycles per second, death was accompanied by a violent rush of blood to fore and hind limbs and tail. These appendages became severely congested and swollen, and so marked was this effect that it was believed possible to use the general macroscopic appearance as a basis for a comparative study of the effects of high frequency oscillations with external heat.

Christie and Loomis[1] in previous experiments with mice maintained that death occurred as a result of "pure" heat. They did not, however, give the amount of heat necessary to duplicate any given high frequency effect, but simply proved that mice were killed in an incubator whose temperature reading was identical with rectal readings of mice killed in the electrostatic field.

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[1] Christie, R. V., and Loomis, A. L., *J. Exp. Med.*, 1929, xlix, 2.

In the succeeding experiments, in order that the rush of blood to the limbs could be more readily observed, rats were selected before the hair coat developed, 7 days being the age of all experimental animals.

Those animals which were killed in an electrostatic field were exposed in an open glass tube between circular plates 9 cm. in diameter at 3 cm. separation. The generating circuit of the apparatus used included a 50 watt, A. C., tube and a transformer of 1100 volts secondary. The coupling to the exposure or auxiliary circuit was made by a single wire feeder, similar to the usual radio antenna feeder. The average output on the exposure circuit was about 3 amperes as measured by a radio frequency ammeter. Saline thermometer readings of this output did not reach blood temperature in the time required for lethal dosages. The rise was at an average rate of 2.1°C. per minute with a total rise in 5 minutes of not over 12°C. above room temperature.

Those animals killed by external heat were subjected until death to the following temperatures obtained in a dry oven: 45°, 55°, 65°, 80°, 95°, 145°, and 160°C. The rats were placed in an open beaker well padded with cotton in order to protect them from contact with the heat of the oven floor and of the container itself.

Body temperatures were taken on all animals at the instant of death. Since rectal readings were impossible due to the small size of the animals, a slit was made in the abdomen just large enough to admit the bulb of the thermometer. The time lapse from death to insertion of thermometer was less than 8 seconds. Temperatures thus obtained were well within the range of rectal temperatures gotten by other workers (Schereschewsky,[2] Christie and Loomis).

At severe exposures to high frequency ranging in output from 3 to 3.5 amperes the rush of blood to the appendages started almost immediately, giving them first a deep pink, then a red, and, finally, as the congestion increased, a dark purple color which persisted after death. All 4 legs became badly swollen, and, the skin being stretched to the breaking point, ruptured on both the tail and the limbs. The appendages gave the appearance of having been burned. The animals sweat profusely in the region of the rump and seemed to suffer intense discomfort before death, which occurred in about

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[2] Schereschewsky, J. W., *Pub. Health Rep.*, 1926, xli, 1939; 1928, xliii, 927.

3 minutes at the higher output. At outputs of 2 amperes death occurred in from 5 to 6 minutes. The characteristic rush of blood to the limbs took place, but more slowly.

In those animals killed in a dry oven at temperatures of 45°C. there was no discernible rush of blood to the limbs and tail, nor was there any congestion. This was equally true at temperatures of 55° and 65°C.

At 80°C., during exposure, the limbs of the animals became highly flushed but this flush seemed to fade out at death. At 95°C. the first real indication of congestion in the limbs and tail was observed. Here, however, there was no swelling but the legs and tail became a deep red in color and this color persisted after death. At 145°C. the fore and hind limbs and tail became congested and swollen, but not to the degree observed in rats killed by high frequencies.

It was only when temperatures reaching 160°C. were used that the effects of external heat were fairly comparable to those effects obtained in electrostatic fields. At 160°C. the congestion was marked and the color of the legs and tail was a deep purple which persisted after death. The hind legs in particular were badly swollen.

Abdominal temperatures taken on rats killed by external heat were essentially the same as for high frequencies, the highest being 45°C. in the case of heat and 45.5°C. in the case of high frequencies.

It is evident here that body regulation is able to keep the abdominal temperature below 46°C., whether the exposure be in an electrostatic field or in "pure" heat, and that neither the abdominal thermometer nor the saline thermometer are measures of the actual heat to which the animal may be subjected when exposed to high frequencies.

Placed between the plates of the auxiliary circuit as a dialectric the animal is exposed to a rapidly oscillating field, a displacement disturbance which manifests itself as internal heat. Christie and Loomis contended that this internal heat may be very high in various parts of the body and this seems to be borne out by the duplication of high frequency effects with external heat.

The degree of external heat necessary to duplicate the high frequency effects here described was found to be not less than 160°C., and that this "pure" heat temperature is very much higher than any measurable heat generated in the electrostatic field.

4999

Adsorption of Staphylococcus Bacteriophage by Serum Globulins.

EARL B. MCKINLEY AND MARGARET DOUGLASS.

From the School of Tropical Medicine of the University of Porto Rico, under the auspices of Columbia University, New York City.

It is well known that bacteriophage may be precipitated from bouillon by alcohol, acetone, and magnesium sulphate. The lytic principle is only slightly impaired by this procedure though length of time of exposure and temperature have been shown to be limiting factors. It is thought that the lytic principle is adsorbed onto protein and hence is naturally carried down with the precipitate. Hauduroy[1] has shown that gelatin, as well as other similar substances, such as agar, gum, etc., will arrest the action of the bacteriophage. Bronfenbrenner[2] states that the influence of agar or gelatin in inhibiting bacteriophagy depends on the fact that these agents prevent water from entering the bacterial cells. De Necker[3] has shown that colloidal metals, bone charcoal, and serum exercise an inhibitory effect upon the bacteriophage and this author thinks that the nature of the adsorption suggests that the lytic principle is a ferment. Angerer[4] has shown that killed Flexner *dysentery bacilli* adsorb about 70% of homologous bacteriophage within 15 minutes. Hauduroy[5] claims to have demonstrated a fixation of the lytic principle by the addition of antidysenteric serum to tubes containing *B. dysenteriae Shiga* and its homologous bacteriophage. In all of these experiments the authors have been dealing with adsorption phenomena. Recently Gay and Holden[6] have shown that a strain of herpes virus (rabbit brain emulsion) when suspended in distilled water may be precipitated with the globulins by bubbling CO_2 gas through the mixture. Resuspension of the globulins in saline when injected intracerebrally into rabbits produced encephalitis. This may also perhaps be regarded as an adsorption phenomenon.

The completeness and thoroughness of such adsorption processes is illustrated by the following experiment with a bacteriophage lytic for *Staphylococcus aureus:*

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[1] Hauduroy, Paul, *Compt. rend. Soc. de biol.*, 1924, xc, 1463.

[2] Bronfenbrenner, J., and Hetler, D., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 480.

[3] De Necker, J., *Compt. rend. Soc. de biol.*, 1922, lxxxvii, 1247.

[4] Angerer, K. v., and Rupp, H., *Arch. Hyg.*, 1928, xcix, 118.

[5] Hauduroy, Paul, *Compt. rend. Soc. de biol.*, 1922, lxxxvii, 966.

[6] Gay, Frederick P., and Holden, Margaret, *J. Infect. Dis.*, 1929, xlv, 415.

The Staphylococcus bacteriophage was titrated and found to be active in 1:10,000,000,000 dilution. One cubic centimeter of this lytic principle was added to 9 cc. of fresh rabbit serum. One cubic centimeter of the mixture was removed for titration and was found to be as active as the original lytic principle. To the remaining 9 cc. of serum-phage mixture 20 volumes of distilled water were added. CO_2 gas was then bubbled through the mixture, following which the material was centrifuged and the globulins were resuspended in 9 cc. of saline made slightly alkaline with a 1% solution of sodium carbonate. The globulin fraction was then tested for lytic activity against the sensitive *Staphylococcus aureus* and the bacteriophage was found to be present in undiminished concentration. Lytic principle, however, could not be detected in the albumin fraction. This phenomenon may be explained upon the basis of the negative charge carried by the bacteriophage and the opposite charge of serum globulin at or below its isoelectric point at pH 5.4 which is reached by treating the serum-phage mixture with CO_2 gas. The isoelectric point of serum albumin is at pH 4.7 and this degree of acidity is not reached by the method employed, the electric charge of the albumin remaining negative, and hence there is no adsorption of the negatively charged bacteriophage by this fraction of the serum.

5000

Further Note on the Salivary Gland Poison of Aëdes Aegypti.

EARL B. MC KINLEY AND MARGARET DOUGLASS.

From the School of Tropical Medicine of the University of Porto Rico, under the auspices of Columbia University, New York City.

In a previous communication[1] we described the poisonous substance contained in the salivary glands of *Aëdes aegypti* and the types of reactions which are produced by introducing this substance intradermally into the human skin. This substance gives rise to a reaction which is practically identical to that following the bite of this insect. The poison is resistant to both freezing and boiling temperatures and contains no hemolytic or anticoagulant substances. It does not produce antitoxin when introduced intravenously into rabbits.

Further studies upon the properties of the salivary gland poison from *Aëdes aegypti* indicate that the poison does not deteriorate on standing for several months. Material tested after 8 months from the time of preparation is just as active as in the beginning. Furthermore the poison may be diluted with 5 volumes of 95% alcohol or ether, desiccated at room temperature over calcium chloride, redissolved in physiological saline, and still retain its full potency when tested by intradermal skin injection. The poison when shaken out several times in ether does not lose any of its strength. Exposure of the salivary gland poison in saline to the action of ultra violet light, at one foot distance, in quartz tubes, for a period of 20 minutes has no apparent effect. The saline extract of the salivary glands gives a negative biuret reaction, a negative Fehlings test but a definitely positive Molisch reaction. The chemical tests indicate the carbohydrate nature of the toxic substance and the skin reactions obtained (wheel, without pseudopods, surrounded by erythema) are quite similar to those obtained with carbohydrates isolated from certain microorganisms.

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[1] McKinley, E. B., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 806.

5001

Resistance of Hemolytic Staphylococci to Bacteriophage Lytic for Non-Hemolytic Staphylococcus Aureus.

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A very common infection in Porto Rico is caused by a hemolytic *Staphylococcus aureus*. These infections are exceedingly virulent as a rule and offer a serious problem in our clinics and hospitals. Pomales[1] has shown that this organism is present in the throat flora of about 19% of supposedly healthy individuals and in pathological cases it predominated in 24%. This organism proved to be the predominating microbe in the crypts and the interior of 65 pairs of tonsils removed at operation. Because of the severity of infections with this organism we have attempted treatment in a few cases with a bacteriophage which is lytic for a non-hemolytic *Staphylococcus aureus*. The administration of the bacteriophage has had no effect upon the course of the infection. The bacteriophage employed was supplied to us by Larkum,[2] who has reported the efficacious use of this lytic principle in clinical cases.

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[1] Pomales, A., *Porto Rico J. Pub. Health and Trop. Med.*, 1929, v, 196.

We have attempted to adapt the bacteriophage which is lytic for a non-hemolytic variety of *Staphylococcus aureus* to 7 strains of hemolytic staphylococci which have been isolated in Porto Rico. Repeated attempts to bring about this adaptation have been made during the past year without success. This is in agreement with the work of Epstein and Fejgin,[3] who have reported the resistance of hemolytic staphylococci to bacteriophage and it may be, as these authors suggest, that such resistance may have some bearing upon the high virulence of hemolytic staphylococci.

5002

Bacteria as "Carriers" of Bacteriophage.

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There has been a great deal of discussion in the literature regarding the production of bacteriophage from bacterial cultures. A classical example of this phenomenon is the *B. coli* of Lisbonne and Carrére[1] which is able to elaborate lytic principle for *B. dysentery Shiga.* This subject has been treated extensively by one of us (McKinley[2]) in another publication. More recently Muckenfuss[3] has studied these cultures supplied to him by us and he concludes that such organisms as *B. coli* Lisbonne may be made lysogenic by exposing the organisms to a bacteriophage and that the organisms so exposed then "carry" the lytic principle and antibodies are produced against the bacteriophage when the "phage infected" bacteria are used for immunization. However, this author states that failure of such antibodies to appear on immunization with bacteria does not necessarily indicate that bacteriophage is not present.

We have attempted to "contaminate" or "infect" a strain of *B. coli* with a bacteriophage lytic for *Staphylococcus aureus.* The organism and lytic principle employed are at wide enough variance to preclude the possibility of any intimate relation existing between them. The lytic principle was active in 1:10,000,000,000 dilution against its sensitive strain of staphylococcus. *B. coli* was sown in bouillon and after 12 hours' incubation 1 cc. of the bacteriophage was added to the culture. Incubation was then prolonged to 24 hours. There was no evidence whatever of lysis, as might be expected. After 24 hours' incubation dilution plates were made from the *B. coli-Staphylococcus* bacteriophage broth culture in order to obtain isolated colonies of the *B. coli*. Typical colonies were picked and sown in broth and incubated over night. This culture was then heated at 58.5°C. for 30 minutes to destroy the microbes and 2 cc. of this heated culture were added to a tube of broth along with an inoculum of the sensitive *Staphylococcus aureus*. This "feeding-up" process was continued through 7 passages and tests for the presence of the bacteriophage against the *Staphylococcus aureus* were made after each passage. After repeated efforts to "contaminate" a strain of *B. coli* in this manner with a lytic principle active against a strain of *Staphylococcus aureus* we have only to report failure to recover the bacteriophage from isolated colonies. The technique described is that which is commonly employed to demonstrate the ability of *B. coli Lisbonne* to elaborate lytic principle for *B. dysentery Shiga*. In the latter case isolated colonies of *B. coli Lisbonne* may be picked and utilized in broth culture to demonstrate that *always* the lytic principle for *B. Shiga dysentery* may be elaborated. We believe that these experiments throw much doubt on the so-called bacteriophage "carrier state" of bacteria though it is perfectly reasonable to suppose that some microorganisms may become *lysogenic* as a result of the influence of a lytic principle upon them. This would not mean a contamination of the organisms with the bacteriophage but rather a process in which the bacteria themselves play a rôle other than a purely mechanical one. This would presuppose some degree of susceptibility (not sensitivity to lysis) on the part of the microbe to the lytic principle and this may be in the nature of a metabolic effect.

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[2] Larkum, N. W., *J. Infect. Dis.*, 1929, xlv, 34.

[3] Epstein, T., and Fejgin, B., *Compt. Rend. Soc. Biol.*, 1926, xcv, 908.

[1] Lisbonne and Carrére, *Compt. rend. Soc. de biol.*, 1922, lxxxvi, 569.

[2] McKinley, Earl B., *Philippine J. Science*, 1929, xxxix.

[3] Muckenfuss, Ralph, *J. Exp. Med.*, 1928, xlviii, 723.

5003

Effects of Low Oxygen Pressure on Respiratory Phenomena.

ROBERT GESELL, CHARLES BRASSFIELD, HUGO KRUEGER, HAYDEN NICHOLSON and MARIE PELECOVICH.

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Eight per cent oxygen mixtures were administered for a period of approximately 30 minutes to 3 dogs anesthetized with morphine and urethane. Such administration produced an initial decrease in oxygen consumption which was followed by an increase slightly in excess of normal. This excess oxygen consumption was ascribed to the increased muscular effort of augmented ventilation. The augmented ventilation increased the elimination of carbon dioxide considerably above normal. On readministration of room air there was a decrease in pulmonary ventilation, a relative retention of carbon dioxide and an oxygen consumption above normal. In one experiment the expiratory quotient rose to 2.86 during the period of oxygen lack and fell on readministration of room air to 0.24. During oxygen lack there was a substantial decrease in the carbon dioxide capacity of the blood which was promptly followed by an increase approaching or exceeding the initial carbon dioxide capacity. There was a similar increase and decrease in blood lactic acid. The blood lactic acid content at the end of the period of oxygen lack was only moderately increased. The lactic acid changes occurred earlier than and were smaller than the changes in blood carbon dioxide capacity. The lactic acid content of the testicle and the rectus femoris muscle before and after the period of oxygen lack was determined. The tissue was dropped in liquid air and a satisfactory suspension was obtained by sectioning in the frozen state with a rotary microtome. The lactic acid content of the testicle and of the muscle was increased during the period of oxygen lack. The amount of lactic acid in the muscle was greater than in the blood. In the testicle it was less than in the blood. This held for both conditions of oxygen plenty and oxygen lack. The hydrogen ion concentrations of the arterial blood, as measured by the quinhydrone electrode, was decreased during lowered alveolar oxygen. On readministration of room air it tended to increase slightly above the normal level. Due to the relatively small increase in the lactic acid content of the testicle and rectus femoris muscle it is tentatively concluded that the acid effect of lactic acid accumulation is exceeded by the alkaline effect of removal of free carbon dioxide associated with increased pulmonary ventilation. Whether the calculations on the acid base equilibrium of resting tissue can be applied to the respiratory center whose activity and metabolism are augmented by oxygen lack remains to be determined by other methods. Until such crucial experiments are performed, however, our present results might be interpreted as indicating a direct stimulating action of oxygen lack independent of increased hydrogen ion concentration.

5004

A Pharmacological Study of Sodium Tetra-iodo-ortho-sulpho Benzoate.

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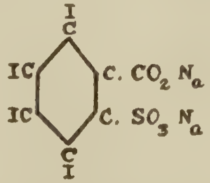
From the Chemical and Pharmacological Research Laboratories, Hynson, Westcott & Dunning, Baltimore.

This substance was synthesized by one of the authors and studied pharmacologically in an intensive and extensive way by the other in connection with a research instituted at the suggestion of an orthopedic surgeon, Dr. Henry Keller, of New York, in quest of a drug suitable for injection into joint cavities for facilitation of x-ray examinations. The compound is a di-sodium salt of tetra-iodo-ortho-sulpho benzoic acid having the following structural formula:



It is a beautiful light yellow crystalline powder freely soluble in water, being practically odorless and tasteless. The molecular weight of this compound is 789.68 and its iodine content, 67.72%. An isotonic solution of the salt requires a concentration of 11.3%, whereas comparative studies on sodium iodide were found to produce isotonic solutions in the concentration of 2.2%. Such an isotonic solution of the new compound contains about 4 times as much iodine as an isotonic solution of sodium iodide and, in addition, is devoid of the objectionable pharmacological features of an inorganic salt solution.

A 10% solution of the salt was made the starting point for all the experiments performed. Such solutions were found to be non-irritating on instillation into the conjunctival sac and application to other mucous membranes. Subcutaneous and intramuscular injections into rabbits, dogs and other animals gave no evidence of local irritation or infiltrations. Intravenous injections of the solution were well tolerated by all higher animals and quantities of 100 mg. per kilo produced no appreciably toxic effect in intact animals. The lethal dose on intravenous injection for rabbits was found to be 0.5 gm. per kilo, for cats under ether anesthesia, 0.5 gm. per kilo, and for dogs under ether anesthesia, 0.4 gm. per kilo. Blood pressure and respiratory tracings studied on the cat and dog showed that after doses of from 100 to 300 mg. per kilo, there was practically no effect on the respiration or the circulation. The lethal doses produced a primary fall in blood pressure which was followed later by paralysis of the respiratory center and last of all by arrest of the heart. The yellow compound, when injected intravenously, is excreted rapidly and almost entirely by the kidneys, only a trace of the compound being found in the bile. On administration of solution by the stomach tube, the compound is also excreted by the kidneys and not at all by the liver. In testing for the presence of the tetra-iodo-ortho-sulpho benzoate in urine and other secretions, the firmly bound iodine cannot be detected by the simple method of using sodium nitrite and shaking out with chloroform, but its presence can be easily demonstrated by first disintegrating the compound by heating almost to dryness, then taking up the residue in water, and testing by the usual chemical methods. Studies on kidney function after intravenous injections of large doses of the compound revealed that it produced no renal irritation.

Studies on isolated tissues showed that the new compound was not depressant for smooth muscle in solutions of 1:1,000. In this connection, moreover, it was noted that it possessed peculiar sensitizing properties for the true sympathetic nerve terminals in that subsequent treatment with epinephrine of such tissues as vas deferens, ureters and uterus gave a potentiated contraction of the muscle as compared with normal controls which were not previously treated with the drug. In view of the benzoate radicle, experiments were made on rabbits to ascertain whether the compound possessed antipyretic properties. No antipyretic effect was noted in animals with artificial fever and, indeed, in normal rabbits a slightly pyretic effect, or an increase in temperature, was produced after intravenous injection of the drug.

Psychopharmacological experiments on albino rats, trained in the circular maze according to the methods of Macht and his collaborators,[1] proved that the compound produced no depression either of the central nervous system or of the neuromuscular mechanism. Phytopharmacological studies, carried out on seedlings of *Lupinus albus,* according to the methods of the senior author,[2] showed that the compound was quite toxic for plant protoplasm although it was little toxic for isolated animal tissues. This was a striking confirmation of the original findings of Macht and Livingston concerning the extreme sensitiveness of plant protoplasm to benzoates.[3]

In view of the pharmacological findings given above and the relatively low toxicity of the drug, clinical observations were justified and were made on selected cases by Dr. Keller in the orthopedic clinic, the results of which are published elsewhere.[4] Dr. Keller found that from 25 to 30 cc. of a 10% solution could be easily injected into the knee joint and gave remarkably well defined roentgenographs revealing structures and conditions around joints which could not be detected by ordinary x-ray examination without previous injections of the drug. Because of the rapid and almost complete secretion of the drug through the urinary system, carefully conducted investigations on selected urological cases are in progress under the supervision of competent genito-urinary specialists. A full description of the chemical and pharmacological studies will be published by the authors later.

5005

Experimental Studies on Visualization of Joints by Injections of a Drug.

HENRY KELLER. (Introduced by David I. Macht.)

From the Orthopedic Department of the Neurological Hospital, New York City.

By the term arthritis the clinician usually means an inflammatory reaction involving the region of a joint. Such an inflammation may involve the synovial membrane alone, when it is termed more strictly synovitis. Usually, however, the inflammatory process involves the articular surfaces proper, their lining membranes, and the musculature and other soft tissues around the joints which are affected directly or indirectly by the patient's condition and play a rôle in the interference with free motion and production of pain and other symptoms. In order to institute rational treatment of a pathological joint condition, it is absolutely necessary to make a definite diagnosis and ascertain the exact condition of the tissues in the joint, or around it, which are involved, namely, whether the disease is confined to the bone itself, to the articular surfaces of the bones, or to the capsular or synovial membrane, and whether it involves the muscular tissue and has produced changes in the connective tissue, leading to adhesions. This is particularly true of the larger joints, and especially of the knee joint, which is the weakest mechanically and is subject to many diseases of acute and chronic nature leading to pathological changes. The most valuable help in the diagnosis of such pathological conditions is roentgenology, which gives a clue to the amount of involvement of bone tissue and the presence of osteophytic outgrowths in the vicinity of the joint. The x-rays, however, are not very helpful in establishing the amount of involvement of the soft tissues in arthritic conditions and, for this reason, any new method which may aid in this connection is welcomed by the orthopedic surgeon.

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[1] Macht and Mora, *J. Pharmacol. and Exp. Therap.*, 1920, xvi, 219.

[2] Macht and Lubin, *J. Pharmacol. and Exp. Therap.*, 1924, xxii, 413.

[3] Macht and Livingston, *J. Gen. Physiol.*, 1922, iv, 573.

[4] Keller, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 852.

The author in 1914 reported experiments on the injection of phenolsulphonphthalein into the joint cavity for the purposes of determining the absorptive power of the joint.[1] Such experiments on the permeability of the synovial membranes have recently been repeated by Curtis and Brunschwig,[2] who have seemingly overlooked the previous work on the subject and made no reference to the paper mentioned above.

In connection with experiments on the injection of phenolsulphonphthalein into the joint cavity, the writer attempted to inject a dye or some other suitable drug into joint cavities to increase the usefulness of x-ray examinations. The ideal conditions for a substance used in such a manner are: (1) it must be comparatively non-toxic; (2) of sufficient density to cast a marked shadow with the x-rays; (3) easily injected and produce the minimum amount of local irritation; (4) easily eliminated a short time after injection. A number of dyes and chemicals have been tried, most of them, however, unsatisfactory. Finally, a new chemical compound, neutral sodium salt of tetra-iodo-ortho-sulpho benzoic acid, prepared by the chemist, Dr. Daniel Twiss, and investigated pharmacologically by Dr. David I. Macht, of Baltimore, was found to fulfil all requirements. This compound is a bright yellow crystalline powder, easily soluble in water, a 10% solution of which is nearly isotonic with blood serum. The compound has a molecular weight of about 750 and its iodine content is 67%. A complete study of the substance is published by Macht and Twiss elsewhere in this journal.[3]

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[1] Keller, Henry, *New York Med. J.*, September 5, 1914.

[2] Curtis, G. M., and Brunschwig, A., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 358.

Using a sterile 10% solution of this drug, the writer has performed experiments, first on dogs, by injecting the solution into the joints, and found that the drug was non-irritating and produced sharply defined shadows, giving a clear picture of the joint cavity with the x-ray. The comparatively low toxicity of the drug having been established by Dr. Macht, solutions of the drug were then cautiously administered for diagnostic purposes to selected patients. The results obtained were very satisfactory. It was found that from 25 to 30 cc. of the 10% solution could be easily injected into the knee joint, and such joints, when examined with x-rays, gave information concerning the condition of the bone surfaces, presence of osteophytes and foreign bodies, and the degree of involvement of the soft tissues not obtainable by a simple x-ray examination. The injection of the tetra-iodo-ortho-sulpho benzoate was found to be particularly useful in detecting the presence and extent of adhesions in and around the joints, especially the knee joint. Injections of the drug did not produce irritation but in some cases mild reactions followed several hours later which, however, did not last long and such reactions in several of the patients led to an improvement in the arthritic symptoms so that the patients desired another injection. It is hardly necessary to add that all the usual surgical and antiseptic precautions must be observed in inserting the needle and making injections into joint cavities. A resumé of the following cases will give an idea of the results obtained. A full account of the experimental and clinical work will be published elsewhere.

Case I. A. K——, aged 40 years, Greek, female. Chronic arthritis of right knee, synovial type. Wassermann negative. Physical examination negative except for right knee. Prior to injections of drug, x-ray examination, anterior-posterior view, showed clear interarticular surface with slight irregularity of inner and upper end of tibia. Lateral view revealed same picture. In popliteal region of knee joint there seemed to be a thickened synovial fold with lime deposits. Injection of 25 cc. of sodium tetra-iodo-ortho-sulpho benzoate, 10% solution, gave the following x-ray findings. Anterior-posterior view: the dye penetrated capsule and synovia over three-quarters of the joint cavity on fibular side; the inner quarter, however, was entirely free from the presence of the opaque drug. Lateral view: the drug invaded all but the popliteal surface and there was also some mid-portion of the capsule lighter than the rest of the joint cavity, thus pointing to the presence of adhesions. Injection of phenolsulphonphthalein showed absorptive integrity of the joint to be good. The patient had a mild reaction after the injection, which cleared up.

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[3] Macht, D. I., and Twiss, Daniel, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 850.

Case II. W. S——, aged 56 years, male, admitted to Neurological Hospital with diagnosis of lues, cerebrospinalis and Charcot's ankles, the right being more involved than the left. Wassermann positive. Lange's colloidal gold test positive. Ordinary x-ray examination confirmed diagnosis of Charcot's disease of the ankles. Twenty-five cc. of 10% solution of yellow dye were injected into right ankle joint. The fluid went in with some difficulty. X-ray examination soon after did not show marked change in contour but the line of demarcation of the debris was more distinct. The loose bodies did not seem to be displaced. The findings, therefore, indicated the presence of loose bodies in the ankle joint, seemingly firmly imbedded in the organized soft tissue exudate. A subsequent operation confirmed the x-ray findings made after injection of the drug.

5006

Mucosal Inclusions in Anterior Gastroenterostomies in Dogs Following Various Methods of Suturing.

KARL H. MARTZLOFF AND GEORGE R. SUCKOW.
(Introduced by George E. Burget.)

From the Department of Physiology, University of Oregon.

The object of this study was to ascertain how healing progressed in anterior gastroenterostomy wounds in dogs when the techniques of suturing employed were similar to those commonly used. The duodenum was sutured to the anterior stomach wall in 50 dogs. The animals were sacrificed by chloroform inhalation after 6, 9, 14, 20, and 27 days. The stomach and duodenum were removed at once and fixed immediately in 10% formalin and were not again disturbed until fixation was complete.

Blocks were taken from the mid-portion of the anterior and posterior aspects of the gastroenterostomy ostium by razor section. Paraffine sections, stained with hematoxylin and eosin, showed in cross section the entire anastomotic zone with the adjacent stomach and intestine. Observations were made on the gross specimens as to amount of infolding at the site of anastomosis as well as size of the ostium. Suture materials were 00 plain catgut and 0 black silk. Five different suture methods were employed in performing the anastomoses for which no gastro-intestinal clamps were used.

We observed distinct variations in wound healing depending upon the suture method used. The outstanding observation of this study, however, was the occurrence of mucosal inclusions in the line of anastomosis as shown in Fig. 1 at *d*.

The mucosa in these inclusions is composed of tubular glands and interglandular stroma. The epithelial lining is columnar with

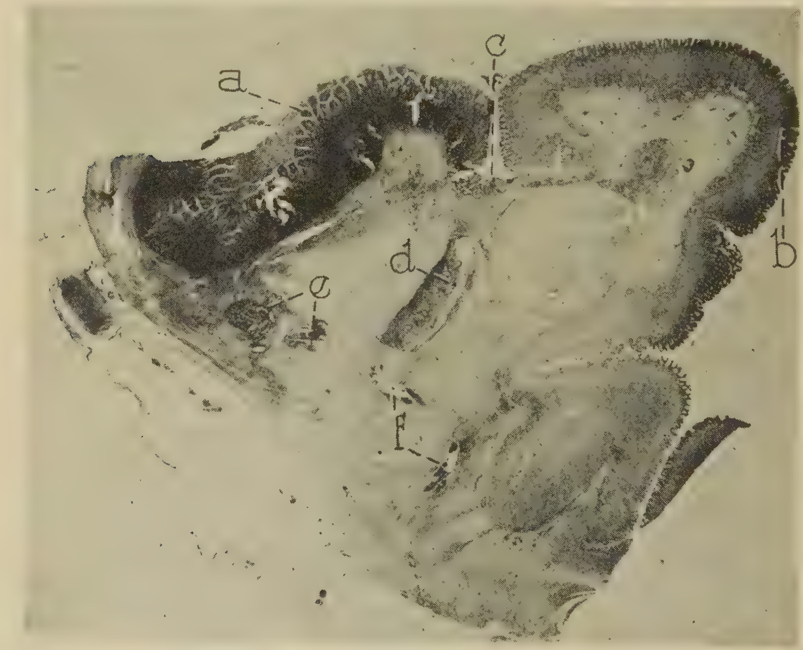

FIG. 1. (Dog 45.)

Low power magnification from anterior aspect of ostium after a 20-day healing period. Technic number 5 was employed. *a*—Intestinal mucosa; *b*—gastric mucosa; *c*—complete mucosal healing at line of anastomosis; *d*—mucosal inclusion in the line of apposition; *e*—mucosal inclusion in wall of intestine associated with a silk suture; *f*—silk suture defects.

coarsely granular basal nuclei. Many of the cells are distended with mucous droplets and mitoses are common. In brief, it has the appearance of viable intestinal mucosa. Serial sections, in so far as they have been studied, have shown no communication between the glandular inclusions and the gastro-intestinal lumen. These mucosal inclusions were observed only where suture methods were used that caused mucosal eversion at the site of anastomosis. They have not, to our knowledge, been described heretofore, and we are now studying their ultimate fate and possible influence on remote wound healing. The mucosal inclusions in the wall of the intestine at *e* are of different origin and have been described.

5007

On the Bactericidal Powers of Lipiodol and Iodipin in Vitro.

JAMES EVERETT BUCKLEY AND ALBERT MATHIEU.
(Introduced by H. J. Sears.)

From the Departments of Bacteriology and Gynecology, University of Oregon Medical School.

A generous literature concerning the use of lipiodol and iodipin in diagnosis reveals the statements by many authors that these iodized oils are antiseptic.

One of us (A. M.) has used these oils in the injection of over 300 gynecologic patients and has occasionally felt some concern regarding the possibility of infection from the material forced through the tubes into the abdominal cavity. No such infection has taken place and we have been led to believe that the oils used actually had antiseptic powers. Nevertheless we felt that some quantitative work to determine more accurately the effects of these oils on bacteria would be of value.

Great difficulties are encountered at once in applying the ordinary methods of disinfectant testing to these heavy oils. Even long continued shaking of specimens of the oils with masses of bacterial growth taken from agar slants gave very poorly dispersed suspensions of the organisms. Visible clumps were invariably present. *Staph. aureus* (a hemolytic strain) and *B. coli* were the organisms used. Inoculations from such imperfect suspensions to broth gave growth in all cases, but since many organisms in the clumps may have been protected from contact with the oil, we did not feel that such tests were trustworthy. Better suspensions of the cultures could be obtained in the pure oils, such as sesame and poppy seed, of which the lipiodol and iodipin are addition products, and subsequent additions of these oily suspensions to the iodized products resulted in somewhat better, though still imperfect, dispersion of the organisms in the latter. Inoculations from these to broth at varying intervals yielded variable results. No growth at all was obtained with *B. coli* and only a portion of the Staph. tubes showed growth. Results with pure poppy-seed oil and sesame oil without addition of the iodized oil gave similar results. Portions of the suspensions dropped from a pipette upon agar plates likewise generally gave negative results. On the other hand, when a drop of the suspension was blown upon a plate in a fine spray, a considerable number of colonies appeared. This was true, both of the *B. coli* and of the *Staph. aureus.* This suggested to us that the intimate mixture of the organisms and the oils had so coated each organism that when it was placed in the medium it was actually kept out of contact with the culture fluid by its film of oil. In the spraying this covering was broken in some cases and growth ensued. Failure of growth in the testing tubes, therefore, indicated, not sterility, but sealing of the organisms away from the medium.

A test of this theory and, we believe, a definite proof of the lack of bactericidal power by these two oils, as well as by the uniodized products from which they are made, is furnished by the following experiment. Several agar plates were inoculated by streaking with saline suspensions of *B. coli* and *Staph. aureus.* A portion of each plate was then covered with the oil to be tested. The plates were incubated for 24 hours right side up. None of the oils showed any inhibitory action upon either organism except iodipin, and here the inhibition was only slight. Since chemical tests of this oil showed a trace of free iodine, it is believed that this substance is responsible for the slight effect noted. With the other substances growth continued to increase for 2 or 3 days in the oil covered as well as the uncovered portion.

We are convinced that no dependence can be placed upon lipiodol or upon iodipin as bactericidal agents.

5008

Effect of Calcium on Cardiac Reactions to Mercury.

WILLIAM SALANT AND HAROLD NAGLER.

From the Biological Laboratory, Cold Spring Harbor, Long Island.

The response of the isolated frog heart to mercury in normal Ringer was first studied by Salant and Kleitman,[1] but as far as we are aware no investigation has been previously reported on the effect of mercury in solutions containing different amounts of calcium. Experiments were therefore carried out with this object in view, Straub's method being used.

After trying different concentrations of mercury, we found that a solution of 1:100,000 in normal Ringer, containing 0.012% calcium chloride, best served the purpose as a basis of comparison. The immediate effect of this amount of mercury was not constant, no changes being observed in most cases, and moderate depression only occurred in some experiments, thus corroborating the observations of Salant and Kleitman, which were made with Simpson's method, modified by his pupil, Livingston. We noticed, however, that in some experiments a gradual decline, especially in the strength of the contractions, occurred, and at the end of one to 3 hours, the amplitude showed a considerable decrease. When the amount of calcium chloride in Ringer's solution was reduced from the normal 0.012 to 0.008 and 0.006% the resistance to mercury was greatly decreased. In a concentration of 1:100,000 (as the chloride) it produced a well marked decrease of the strength of the contractions, the effect setting in after a short latent period, and within 3 or 4 minutes the amplitude was reduced in some experiments 50% or more. The rate was also decreased but to a much smaller extent. Drastic reduction in the strength of the contractions was observed in some of these experiments. The effect occurred almost immediately after the introduction of mercury, the depression increasing rapidly and after one minute the contractions became very feeble. As before, the rate was much less affected than the force of the contractions. The greatly increased toxicity of mercury was practically the same whether the amount of calcium chloride was 0.006 or 0.008%. The same amount of mercuric chloride in Ringer's solution containing 0.015% calcium chloride also caused depression of the heart, but the process was much slower than in the presence of deficient amounts of calcium. The rapid, almost violent, decrease in the force of the contractions which was sometimes produced by small amounts of mercury and subnormal amounts of calcium never occurred after treatment with the same concentration of mercury when the calcium content was increased beyond 0.012%. The difference in the effect was even more pronounced in the experiments in which the calcium chloride content was increased to 0.02%. In one experiment the heart, though weak, was still contracting 50 minutes after treatment with mercury 1:100,000. The same amount of mercury in the other experiments produced considerable slowing, but no change in the strength of the contractions could be noticed, and at the end of 21 hours feeble beats were still observed. The antagonistic effect of calcium was less pronounced, however, when the concentration of mercuric chloride was increased. Solutions of 1:50,000 were almost equally potent when the amount of calcium chloride was 0.012 or 0.2%. Heart action continued 15 to 20 minutes, and in one experiment with mercury and normal Ringer the heart was still beating 45 minutes after the introduction of mercury. But when the amount of calcium was subnormal mercury 1:50,000 promptly brought the heart to a standstill.

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[1] Salant and Kleitman, *J. Pharmacol. and Exp. Therap.*, 1922, xix, 315.

The results presented above justify the conclusion that deficient amounts of calcium greatly increase the resistance of the frog heart to mercury. While excess calcium may be antagonistic to mercury, this is less certain than the injurious effects produced when the heart is exposed to mercury in the presence of subnormal amounts of calcium.

5009

Vitamin Requirements of Nursing Young. VIII. Effect of Vitamin B* Deficiency on Alkaline Reserve of Blood of Nursing Young of Albino Rat.†

BARNETT SURE AND M. C. KIK.

From the Department of Agricultural Chemistry, University of Arkansas, Fayetteville.

In order to determine the influence of uncomplicated vitamin B deficiency on the alkaline reserve of the blood of nursing young of the albino rat, 84 nursing young, whose mothers received stock diet No. 6‡ were sacrificed during the later part of the lactation period, and the carbon dioxide volume per cent determined on blood secured from the carotid artery. We used the manometric method of Van Slyke and Neill[2] on samples of 0.1 cc. of plasma.

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* Unless stated otherwise, vitamin B deficiency refers to the uncomplicated polyneuritis produced by a deficiency of only the antineuritic factor. An abundance of the antipellagric factor was provided by an ample supply of autoclaved yeast in the maternal diet.

† Research paper No. 142, Journal series, University of Arkansas.

Since in the pathological nursling there has been, because of stunting of growth, a considerable prolongation of the nursing period, 6 additional animals on maternal stock diet No. 6, 32 days of age, were taken during the post-lactation period. The range of carbon dioxide volume per cent found was 37 to 65.

The 72 pathological animals examined were in a condition of prolonged maintenance, or in a condition of prolonged maintenance accompanied by incipient polyneuritis. The avitaminosis of the nurslings was produced on maternal diet 1438,[3] adequate in every respect with the exception of vitamin B. Only 4 pathological animals showed a deviation from the normal which occurred during the last stages of polyneuritis. These results are in agreement with the findings of Sure and Smith[4] on growing and adult rats.

5010

Vitamin Requirements of Nursing Young. IX. Effect of Vitamin B Deficiency on Glycogen Content of Liver of Nursing Young of Albino Rat.*

BARNETT SURE AND MARGARET ELIZABETH SMITH.

From the Departments of Agricultural Chemistry and Home Economics, University of Arkansas, Fayetteville.

In this study we have employed 50 nurslings, 29 control and 21 pathological. The animals were sacrificed by cutting both carotid arteries with a sharp scissors. The vagus nerves were also severed and death occurred almost instantaneously. The chemical method used for determination of glycogen of the livers was one kindly furnished us by Dr. C. F. Cori, which is a modification of the

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‡ Stock diet No. 6 is a modification of our stock diet No. 1 (1) in which 5% of the corn is replaced by an equivalent amount of rice polishings.

[1] Sure, B., *J. Biol. Chem.*, 1926, lxix, 65.

[2] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, lxi, 523.

[3] Sure, B., and Smith, M. E., *J. Nutr.*, 1929, i, 537.

[4] Sure, B., and Smith, M. E., *J. Biol. Chem.*, 1929, lxxxiv, 727.

* Research paper No. 143, Journal series, University of Arkansas.

Pflueger method[1] used by Cori and Cori in their studies on "The Fate of Sugar in the Animal Body."[2] In the case of normal animals and those animals whose livers contained appreciable amounts of glycogen, the Folin-Wu method[3] of determining apparent sugars was used. In cases where the livers contained very small amounts of glycogen we substituted the Folin micro-ferricyanide method.[4] The glycogen content of the livers was expressed in terms of glucose, *i. e.,* total reducing sugars.

It was found that the glycogen content of the livers of the nurslings on maternal stock diet No. 1,[5] which is the control ration, varied from 1300 to 1600 mg. of glucose per 100 gm. of liver. On the other hand, the glycogen content of the livers of the polyneuritic nursing young[6] showed a range of 20 to 110 mg. of glucose per 100 gm. of liver. Expressed per 100 gm. of body weight, the glycogen content of the control animals showed a range of 45 to 56 mg. of glucose; whereas, the pathological group showed a range of 0.8 to 6.2 mg. of glucose. The marked decrease in the glycogen content of the liver is the most pronounced chemical change we have found to date in nursing young suffering from uncomplicated vitamin B deficiency.

5011

Vitamin Requirements of Nursing Young. X. Effect of a Deficiency of Vitamin B Complex and Vitamin B on Histological Changes in Liver of Nursing Young of Albino Rat.*

BARNETT SURE, DOROTHY J. WALKER AND HARVEY S. THATCHER.

From the Laboratory of Agricultural Chemistry, University of Arkansas, Fayetteville, and the Laboratory of Medical Pathology, University of Arkansas, Little Rock.

A total of 303 animals were employed for this work, 156 on control diets, 67 on a maternal diet deficient in the vitamin B complex,[1] and 80 on a maternal diet deficient in vitamin B.[2] The fixatives used were Bouins and formalin. A number of tissues were stained with Delafield's hematoxylin and eosin while others were stained with iron alum hematoxylin and eosin. Frozen sections were also made of a representative number of the livers and stained with Sudan III.

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[1] Pflueger, E., in Abderhalden, E., *Handbuch der biochemischen arbeitsmethoden,* Berlin and Vienna, 1910, ii, 1070.

[2] Cori, C. F., and Cori, G. T., *J. Biol. Chem.,* 1926, lxx, 557.

[3] Folin, O., and Wu, H., *J. Biol. Chem.,* 1920, xli, 367.

[4] Folin, O., *J. Biol. Chem.,* 1928, lxxxi, 231.

[5] Sure, B., *J. Biol. Chem.,* 1926, lxix, 65.

[6] Sure, B., and Smith, M. E., *J. Nutr.,* 1929, i, 537.

* Research paper No. 144, Journal Series, University of Arkansas.

[1] Sure, B., *J. Nutr.,* 1928, i, 139.

Compared with the controls, the majority of the nursing young whose mothers received a diet deficient in the vitamin B complex showed fatty metamorphosis of the liver, as indicated by marked vacuolation. The fact that there was an actual increase in the weights of the livers of such pathological nurslings as well as considerable increase in the weight of liver in proportion to the body weight of such nursing young, suggests that the pathological condition encountered is fatty infiltration rather than fatty degeneration. On the other hand, the nursing young suffering from uncomplicated vitamin B deficiency showed no demonstrable fatty metamorphosis in the liver.

5012

Experimental Production of Deciduomata in the Rat by an Extract of the Corpus Luteum.

W. O. NELSON AND J. J. PFIFFNER. (Introduced by E. Ponder.)

From New York University and the Biological Laboratory, Cold Spring Harbor.

That Loeb's hypothesis concerning the production of deciduomata in experimental animals is correct has become increasingly apparent. He concluded following his work upon guinea pigs[1] that this reaction depends upon the continued presence of the hormone of the corpus luteum. Frank[2] showed that this phenomenon can be induced in lactating rats by the stimulation of the uterine endometrium at the proper time following parturition. Long and Evans[3] confirmed and extended his findings. In their work pseudo-pregnant as well as lactating animals were employed. They also introduced a convenient and non-traumatic method of uterine stimulation, *viz.,* the insertion of silk threads through the lumen of the uterus. Evans and Simpson[4] were able to demonstrate the efficacy of both the growth and the maturity hormones of the anterior pituitary in evoking a uterine response. The result depended upon the induction of a pseudo-pregnant condition in the host's ovaries. Parkes[5] employing an alkaline extract of the anterior pituitary showed that the phenomenon could be induced in the mouse.

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[2] Sure, B., and Smith, M. E., *J. Nutr.*, 1929, i, 537.

[1] Loeb, L., *J. Am. Med. Assn.*, 1909, liii, 1471.

[2] Frank, R. T., *Surg. Gyn. and Obst.*, 1911, xiii, 36.

[3] Long, J. A., and Evans, H. E., *Memoirs of Univ. of Calif.*, 1922, vi, 84.

Following this evidence for the rôle played by the corpus luteum in placentoma formation it was but a step to the employment of active extracts of corpora lutea in creating a condition in the uterus whereby the reaction could be called forth. Weichert,[6] using Hisaw's preparation was able to produce deciduomata in spayed and normal animals and Goldstein and Tatelbaum,[7] employing the extract prepared by Allen and Corner, were able to confirm Weichert's results on spayed guinea pigs.

It was considered of value to demonstrate the reaction in the rat when evoked by suitable treatment with an active extract of the corpus luteum. We were also interested in determining the activity of the preparation employed by us in sensitizing the uterus. Previously this extract has been shown to be active in maintaining pregnancy in the absence of the ovaries (Harris and Pfiffner[8]) and in developing the mammary glands (Nelson and Pfiffner, unpublished data). Full details concerning the nature of this extract will be published in a later paper. A related preparation has been employed in the inhibition of oestrus (Haterius and Pfiffner[9]) and in the prolongation of normal pregnancy (Nelson, Pfiffner and Haterius[10]). The latter extract has been described in previous publications.

Both normal and spayed animals were employed (12 in all). In the series of normal females (4) injection of the extract was begun during oestrus. In the spayed series the animals (4) were oöphorectomized during oestrus and injection commenced the same day. The extract was introduced subcutaneously and varied in quantity from 0.2 to 0.25 cc. (1.0 cc. equivalent to 40 gm. fresh corpora lutea) daily throughout the experiment. From 3 to 5 days follow-

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[4] Evans, H. E., and Simpson, M. E., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 597.

[5] Parkes, A. S., *Proc. Roy. Soc., B.*, 1929, civ, 183.

[6] Weichert, C. K., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 490.

[7] Goldstein, L. A., and Tatelbaum, A. J., *Am. J. Physiol.*, 1929, xci, 14.

[8] Harris, R. G., and Pfiffner, J. J., *Anat. Rec.*, 1929, xliv.

[9] Haterius, H. O., and Pfiffner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 818.

[10] Nelson, W. O., Pfiffner, J. J., and Haterius, H. O., *Am. J. Physiol.*, 1930, xci, 690.

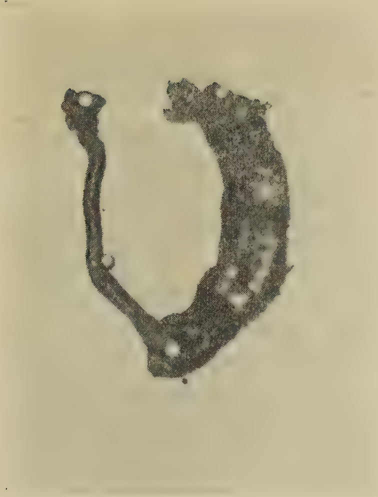

FIG. 1.
Stimulated and control horns of a treated animal. The remnants of two of the threads may be seen.

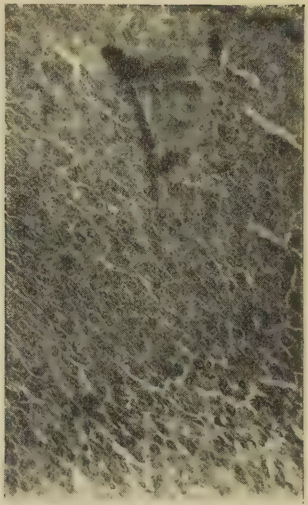

FIG. 2.
Microphotograph of a cross section through a deciduoma. X 90.

ing the initial injection the animals were operated and 3 or 4 silk threads introduced into one horn of the uterus. They were sacrificed from 3 to 7 days later and the uteri recovered for histological study.

In every instance deciduomata were present, the largest (8-10 mm.) being found in the animals in which stimulation occurred on the third day of the experiment and the sacrifice 7 days later. Fig. 1 shows the marked response called forth by stimulation of the endometrium under these conditions. The control horn had been subjected to exactly the same influences except the slight stimulus afforded by the threads. Fig. 2 shows the decidual cells in a cross section through a deciduoma.

The conditions essential for placentoma formation are (1) the uterus must be prepared for the luteal stimulus by the action of the follicular hormone upon the endometrium—a priming action, (2) the uterus must be sensitized by the lutein hormone for several days prior to stimulation and must be under its influence throughout the life of the deciduoma. The incidence of oestrus results in the disappearance of the tumor. As may be noted the animals employed in this work complied with these qualifications, oestrus occurring in each case on the day of initial injection and not again appearing throughout the course of the experiment. The importance of the preparatory action of oestrus was apparent from the failure of one animal to respond. She was treated in every way as the other injected animals, except that she had been spayed 6 weeks previously and hence her uterus had not been primed by the follicular hormone.

As a control on any possible traumatic effect of the stimulus, a series of untreated rats were operated and threads drawn through the uteri at the appropriate time. They were examined at the onset of the next oestrus a few days later. In no case was any effect of the stimulus found, either upon the oestrous cycle or upon the uterus.

It is to be concluded that under the proper conditions placentomata may be induced in normal and spayed rats by treatment with a lipoid extract of the corpus luteum. Either this extract or a related one has previously been demonstrated to be active in fulfilling the other known functions of the corpus luteum.

5013

Calcification of Arteries in Rats fed Viosterol.

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Indications that irradiated ergosterol administered perorally causes deposition of calcium in the arterial walls have appeared in the literature. Hückel and Wenzel[1] obtained thickening of the anterior portion and roughening of the inner surface of the aorta in rabbits. Hermann[2] found weight loss and evidence of calcification in the kidneys in rats. Hess[3] found hypercalcemia in infants.

Experimental production of calcification of arteries in laboratory animals such as the rat, which does not normally show this condition, would afford material for study of possible influences which hasten, retard, or remove the deposition.

Methods and Results. Four adult male albino rats were placed in separate cages, and fed a balanced basal ration. To each of 3 of these 1.5-2.0 cc. Viosterol (Irradiated ergosterol 100 D in oil) mixed in cheese or bread crumbs was fed daily. The fourth rat was fed a like quantity of cheese and oil. The hair became rough and coarse in the Viosterol fed rats within about 10 days and was shed gradually until the skin could be seen. The animals appeared restless and lost weight until at the end of 50 days their weight was reduced 50%. One of the animals died after 55 days, one after 67 days, and the third was killed after 74 days. X-ray examination of the third animal showed a definite shadow in the position of the aorta from its beginning throughout the thorax and abdomen. Macroscopic examination confirmed the X-ray findings, dense annular calcification being evident even to the branches of the iliacs. Similar examination of the other animals revealed the same findings but in less degree. Microscopic examination of areas of the aortae showed the intima to be the chief site of deposition with involvement of the media in the most extensive and thickly calcified areas. The control rat was in healthy condition and showed a normal arterial system at conclusion of the experiment. Further study is in progress.

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[1] Hückel and Wenzel, *J. Am. Med. Assn.*, 1929, xciii, 1427.

[2] Hermann, Siegwart, *Klin. Wochensch.*, 1929, viii, 1752.

[3] Hess, A. F., Lewis, J. M., and Rivkin, H., *J. Am. Med. Assn.*, 1928, xci, 783.

5014

A Direct Method for the Estimation of Venous Blood Pressure.

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(Introduced by Samuel R. Haythorn.)

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After an examination of the methods in vogue for the determination of the pressure of the venous blood and after a consideration of the difficulties involved in their use, we have succeeded in measuring the venous pressure by inserting a needle into the median basilic vein and observing the rise of the blood in a vertical tube. This simple and direct procedure was first used in 1735 by Stephen Hales, a British clergyman, who inserted tubes into the crural artery and the jugular vein of a mare and observed both arterial and venous pressures. The method has since been neglected, 2 other procedures being now in general use. The indirect, tambour method of Eyster and Hooker[1] is effective only when the veins are distinctly visible.

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[1] Hooker, D. R., and Eyster, J. A. E., *Bull. Johns Hopkins Hosp.*, 1908, xix, 274.

It is subject to large personal error and the time required for an observation is appreciably longer. The direct method of Moritz and Tabora[2] is complicated by the use of an additional fluid, *e. g.*, salt solution. Moreover the results do not appear to be entirely reliable.

The apparatus* which we have used, Fig. 1, is an L-shaped pyrex glass tube, ground at the horizontal end to fit an 18 gauge hypodermic needle. The vertical portion of the tube has an internal diameter of 4 mm. and is 30 cm. in length. The readings are taken with the help of a celluloid metric scale which is fastened to the vertical portion of the tube by means of spring clips. The capillary rise of the blood in such a tube is approximately 0.5 cm. and may cause a positive error of this magnitude in the determination. If the pressure is to be related to a column of water, the specific gravity of the blood will introduce an error in the opposite direction. Since the average specific gravity of blood is about 1.06, these errors are balanced when the venous pressure is equal to 10 cm.

A consideration of the capillarity involves also the question of the ease of wetting the glass by the blood. It would seem more satisfac-

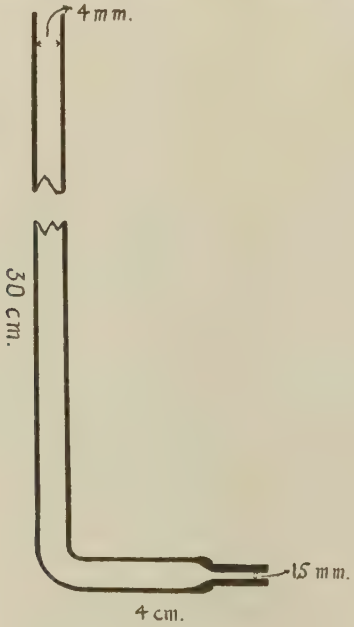

FIG. 1.
Venous pressure manometer tube.

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[2] Moritz, F., and Tabora, D., *Deutsche Arch. f. klin. Med.*, 1910, xcviii, 475.

* The tube has been made for us by the W. J. Gilmore Drug Company, Pittsburgh, Pa.

tory, perhaps, to ignore both these factors and let our reading of the venous pressure represent merely a column of blood without further connotations. The error in obtaining the figure is probably never more than 0.5 cm.

The estimation is made with the patient in the dorsal position, Fig. 2, assumed 15 minutes prior to the reading. The relaxed arm is extended parallel to the mid-axillary line and is supported by a pillow. The veins of the arm are then approximately parallel to the course of the vena cava and possible compression of the veins by contracted muscles is avoided. It would, of course, be desirable to have the inner aspect of the elbow at the level of the right auricle, but to achieve such a position with accuracy is practically an impossibility. We believe, therefore, that, if the arm is always placed in the mid-axillary line a satisfactory basis for comparative readings is furnished. Any constricting clothing should be removed. The cuff of a standard sphygmomanometer is then loosely applied to the arm well above the elbow and the skin at the bend of the elbow cleansed with iodine and alcohol. Slight constriction of the arm is now made by raising the cuff pressure to 10 or 15 mm. of mercury. The needle of the venous pressure manometer is inserted and the

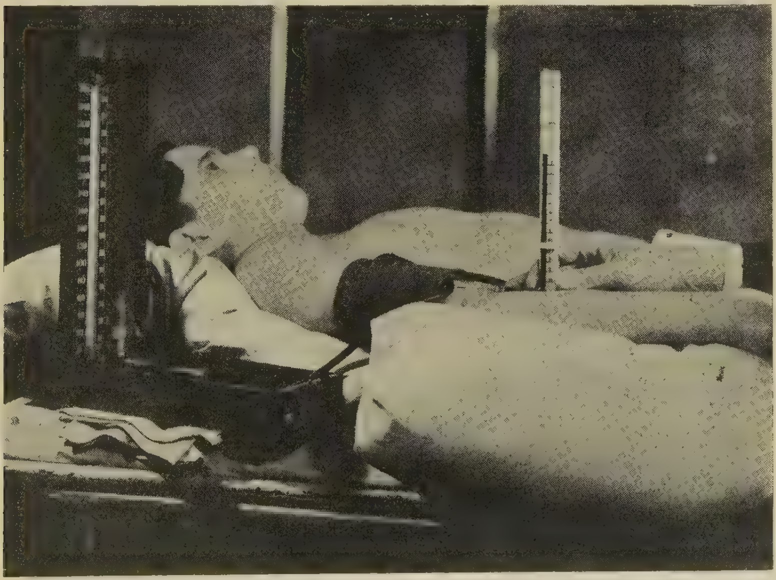

FIG. 2.

Method of observing the venous pressure. It will be noticed that the sphygmomanometer does not register zero. This is due to a fault in the instrument and not to inflation of the cuff.

cuff pressure immediately released by opening the outlet valve of the sphygmomanometer. The constriction by the cuff is thus removed before the blood column has reached the elbow of the manometer. The entire procedure, including constriction of the vein, insertion of the needle and deflation of the cuff, is completed within a few seconds.

The blood rises in the manometer tube at a progressively decreasing velocity until it becomes stationary or pulsates slightly with the cardiac rhythm. The reading is then taken. Finally the cuff is slightly inflated. If the blood rises in the tube, the patency is assured. The cuff is again deflated and the needle immediately re-

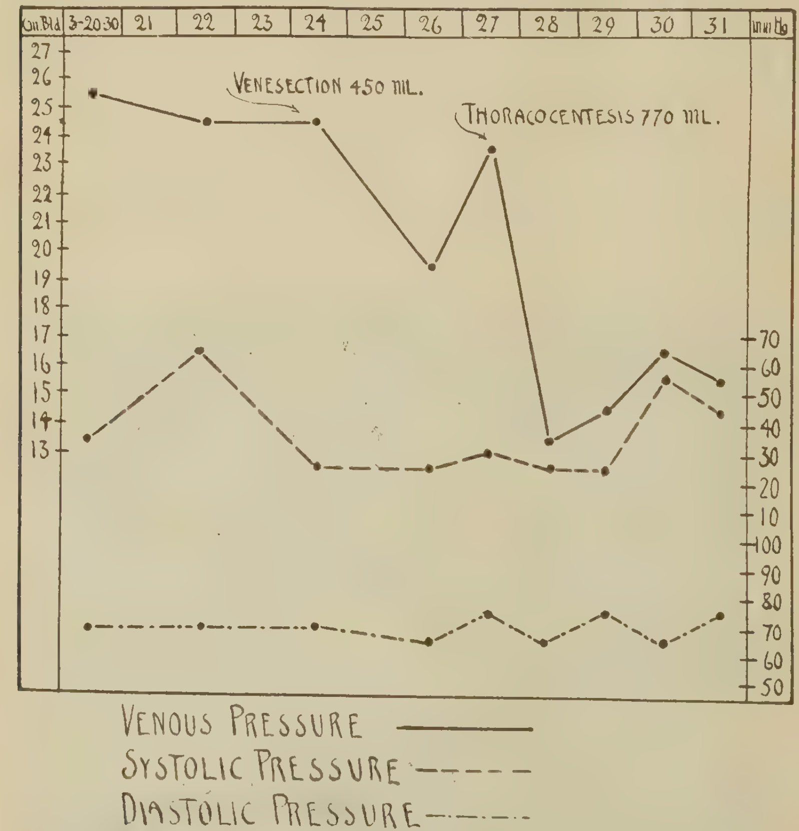

FIG. 3.

The appended graph is that of a case of congestive heart failure, showing the results on venous pressure produced by venesection and subsequent thoracocentesis. The readings were made by the direct manometer. The first 3 readings (taken by 2 different observers) are practically uniform. Following the removal of 450 ml. of blood there is a drop to 19.5 cm. with a prompt subsequent rise. A further and more decisive drop is seen following the removal of 770 ml. of fluid from the left pleural cavity.

moved to prevent a return flow of blood. The site of the puncture is then cleansed with alcohol. The entire procedure requires little more time than an average estimation of arterial blood pressure. We have noted no major technical difficulties nor significant sources of error.

The venous pressure in a series of 40 normal individuals varied between 4 and 10 cm. with most of the figures ranging between 6 and 8 cm. The determination has been applied largely to patients with hypertension, pneumonia or congestive heart failure. It has not seemed necessary to take readings oftener than once daily, except in the most severe cases, in which a second observation has been made only after venesection or after the removal of pleural transudate. Otherwise observations have been made at intervals of 1, 2 or 3 days.

In a series of over 200 readings, we have not been confronted by any difficulties from traumatism by the needle and have not been obliged to resort to other veins than those at the elbow. The amount of blood lost during a reading—1-3 ml.—is negligible. We have not encountered any instances of clotting in the vein. Our experience leads us to believe that this method possesses simplicity, reasonable accuracy and freedom from error of a personal nature.

We are grateful to Dr. A. J. Bruecken for the preparation of the photograph and to Mr. C. F. Thilo for the drawings.

5015

Utilization of Calcium Soaps by the White Rat.

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The formation of calcium soaps in the intestine has been accepted by many authors as being fatal to calcium and fatty acid absorption (Givens,[1] Bosworth,[2] Telfer,[3] *et al.*). On the other hand Holt, Courtney and Fales[4] found evidence that calcium was better assimilated by infants when the diet contained a liberal amount of fat.

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[1] Givens, M., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxi, 421.

[2] Bosworth, A. W., Bowditch, H. I., Giblin, L. A., *Am. J. Dis. Children*, 1918, xv, 397.

[3] Telfer, S. V., *Quart. J. Med.*, (Oxford), 1922-23, xvi, 45.

[4] Holt, E., Courtney and Fales, *Am. J. Dis. Children*, 1918, xvi, 52.

Oelsner and Klinke[5] have demonstrated that ox-gall *in vitro,* readily dissolves a mixture of calcium soaps obtained from the insoluble fatty acids of butter fat. They further found that these calcium soaps were converted into sodium soaps by the action of sodium carbonate and bicarbonate, varying in amount from 8.6% to 19.5% under the conditions of concentrations used and at 37°C. That the bile salts play an important rôle in the absorption of fatty acids and their calcium soaps was shown by Adler.[6]

We have studied the utilization of various calcium salts, using the white rat as the experimental animal. The length of the periods was 18 days in most instances.

The ration fed consisted of: Casein, 18%; mineral salts, 3%; sucrose, 35%; starch, 36%; calcium soap, 4 or 8%. Two-tenths of a gram of yeast and two drops of cod liver oil were given daily. The casein was a low calcium product (0.019% CaO) obtained from the Harris Laboratories, Tuckahoe, N. Y.; Osborne-Mendel[7] salts, modified by the omission of calcium, supplied the other minerals. The calcium soaps were prepared by the method of Harrison[8] from fatty acids of tested purity obtained from the Eastman Kodak Co. The mixed soaps used were prepared from the mixed fatty acids of lard. There was very little neutral fat in the ration, most of which came from the cod liver oil and the yeast, except where lard (10%) was added to the calcium palmitate and the mixed soap rations. The rats used were males about 35 days of age weighing approximately 65 gm. each. The neutral fats, fatty acids and soaps of the feces were determined by the modified Roese-Gottlieb method, as used by Holt, Courtney and Fales.[9]

If calcium soaps are absorbed as undissociated salts there should be perfect agreement between the rates of absorption of the fatty acid radicle and that of the calcium atom. This perfect agreement seems to be substantially found with calcium oleate where both the calcium soaps and the fatty acid radicle were absorbed to the extent of about 90%. With calcium stearate, however, the situation was different, the calcium soaps of the feces being about 30% less than that of the free fatty acid. However, if we assume that the feces contained 30 mg. of free fatty acids per day derived from bile, bacterial residues, etc., then the utilizations of the calcium and the stearic acid in calcium stearate appear to be equal. Since the feces contained about 30 mg. of free fatty acids per day when the diet contained no calcium soaps it seems reasonable to assume that approximately that much fecal free fatty acids had an origin other than from the calcium soaps of the diet. On the whole, therefore, our results are in agreement with the idea that calcium soaps are absorbed without undergoing previous decomposition or hydrolysis. Calcium butyrate, being very soluble was readily assimilated. Since the feces on this diet contained large quantities of calcium carbonate, it is likely that the calcium, above the requirements for bone formation, was excreted into the intestine as the carbonate. The urine after feeding calcium butyrate showed a relatively high calcium content.

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[5] Oelsner, A., Klinke, K., *Jahrb. Kinderheilk,* 1928-29, cxxii, 58.

[6] Adler, E., *Arch. Verdauungs-krankh.,* 1927, xl, 174.

[7] Osborne, T. B., Mendel, L. B., *J. Biol. Chem.,* 1918, xxxiv, 131.

[8] Harrison, G. A., *Biochem. J.,* 1924, xviii, 1222.

[9] Holt, E., Courtney and Fales, *Am. J. Dis. Children,* 1919 xvii, 38.

The storage of calcium on the calcium lactate ration was 51 mg. CaO per rat per day; on calcium butyrate, 41-45 mg.; on calcium palmitate, 26-35 mg.; on calcium oleate, 24-32 mg.; and on calcium stearate 16-23 mg. per day.

Conclusion. Calcium palmitate soap was utilized to the extent of 38 to 66%; calcium oleate 90 to 91%; mixed calcium soaps from lard, 70-75%. Calcium stearate was rather poorly utilized, 25 to 45% of the soap disappearing from the intestinal contents.

5016

Experimentally Induced Intermenstrual Bleeding in Ovariectomized Monkeys.

WILLIAM PAUL MADDUX. (Introduced by Edgar Allen.)

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Recently Hartman[1, 2] has reported the occurrence of intermenstrual bleeding in the monkey *(Macacus rhesus)*. This usually appears between the 11th and 14th days of the menstrual cycle. Ovulation in the monkey, as checked by recovery of ova from the uterine tubes, occurs between the 10th and 14th days (Corner[3] and Allen[4]). By means of exploratory laparotomy Hartman was able to correlate this phenomenon with changes in the ovary. His observations led him to believe that this interval bleeding was a nor-

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[1] Hartman, Carl G., *Science*, 1928, lxviii, 452.

[2] Hartman, Carl G., *J. Am. Med. Assn.*, 1929, xcii, 1992.

[3] Corner, George W., *Carnegie Pub.*, Contrib. to *Embryol.*, 1923, xv, 75.

[4] Allen, Edgar, *Carnegie Pub.*, Contrib. to *Embryol.*, 1927, xix, 1.

mal occurrence correlated with ovulation, or with the advanced development of one or more large follicles which, however, might fail to culminate in ovulation.

The present experiment deals with 2 adult ovariectomized macaques which were treated with ovarian hormone* in the form of gelatin pessaries for intra-vaginal administration. Daily doses were administered for 12 days. The beginning daily dose of 20 rat units was gradually increased until during the last 3 days of the experiment each animal was receiving 40 rat units per day. A total of 375 rat units of hormone was administered to each animal.

Daily examinations of vaginal contents were carried out by Hartman's method during the time of hormone administration and continued for 7 days following cessation of treatment. A microscopic study revealed the usual cellular content as described by Corner[3] and by Allen.[4] On the 10th, 11th and 12th days of the treatment and on the first day following the cessation of the treatment erythrocytes were demonstrated in the vaginal contents. In the cases of these 2 ovariectomized monkeys this ovarian hormone successfully substituted for the endocrine function of the ovaries in inducing this phenomenon experimentally.

5017

A Note on the Use of Wheat Starch in "Synthetic" Rations.

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In the preparation of vitamin-free synthetic rations for animal experiments the purification of ingredients is a time consuming task. A starch which does not seem to need purification for vitamin B work may be of interest. Wheat starch, a by-product in the manufacture of gluten, was found to be considerably cheaper than the best grade of pure corn starch and entirely lacking in the antineuritic factor. Over 60 rats were used in making the comparisons and checking the results reported here. Polyneuritis developed in from 3 to 4 weeks when a ration containing wheat starch, purified casein, salt mixture, crisco, and cod liver oil was supplemented with 0.5 gm. daily of autoclaved yeast. There was no apparent difference between the response on the raw starch and on that which had been autoclaved for 3 hours at 18 lbs. pressure when food intake was limited to the same amount for all rats. In a preliminary series of tests the rats receiving the synthetic ration containing autoclaved starch made a greater temporary gain and were slower in developing polyneuritis than the ones on the raw starch ration. Since this could not have been due to a difference in vitamin content it was puzzling until it was noted that the rats ate more of the autoclaved than of the raw starch ration. With limited food intake the growth in the 2 groups was the same, indicating that the difference must have been one of palatability and not of availability or vitamin B content. When raw yeast was used as a supplement, normal growth resulted with either the raw or autoclaved starch rations. No effort was made to determine the vitamin G content of the wheat starch, but it is unlikely that it carries any appreciable amount.

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* "Amniotin" supplied by E. R. Squibb and Sons.

These data seem to indicate that wheat starch is a satisfactory and economical substitute for corn starch in synthetic rations and that it does not require autoclaving to insure its freedom from the antineuritic factor.